CONTRACEPTIVE AND ENDOMETRIAL ASPECTS OF PROGESTINS AND ANTI-PROGESTINS

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Declaration

I declare that this thesis has been composed by myself, and the studies presented here are the results of my own independent investigations, unless otherwise acknowledged.

The content of this thesis has not and is not being currently submitted for candidature for any other degree, diploma or professional qualification.

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Abstract

The main focus of the studies undertaken as a part of this thesis was to develop new methods of contraception, while broadening our current understanding of the anti-fertility potentials of two progesterone receptor modulators: antiprogesterone compound mifepristone, and the synthetic progestin Levonorgestrel (LNG). We suggest that LNG taken immediately before ovulation acts as an emergency contraceptive (EC) by delaying or preventing ovulation. We have demonstrated that the combination of a home-use fertility monitor with once a month administration of mifepristone (especially if mifepristone is administered at the early luteal phase) is an acceptable contraceptive option with minimal side effects. Our investigations have explained some characteristics of non-compliant behaviour. We have shown that the microelectronic monitoring systems provide objective information no other monitoring technique can produce. This information offers the opportunity to make the optimum use of potentially effective treatments while validating research evidence. We have further illustrated, that this microelectronic system of Luteinizing Hormone (LH) detection can replace laboratory assays in monitoring frequently changing hormone levels in contraceptive research because it is easy to use and provides reliable data on compliance. Finally, we conclude that mifepristone induce endometrial-shedding & vaginal bleeding, in the mid-luteal phase by a mechanism involving both Prostaglandin Dehydrogenase (PGDH) and Cyclo-oxygenase – 2 (COX-2) to increase local Prostaglandin (PG) levels in the endometrium. These studies provide information which will be useful in the development of novel methods of contraception involving a "once a month" pill.

Abbreviations

AA	Arachidonic acid
ABC	Avidin Biotin Peroxidase Complex
ABC-HRP	Avidin Biotin Peroxidase Complex – horseradish peroxidase
ALT	Alanine amino transferase
AR	Androgen receptor
COC	Combined oral contraceptives
COX-2	Cyclo-oxygenase - 2
CPEFM	ClearPlan Easy Fertility Monitor
cPR	Chicken PR
CVF	Cervico-vaginal fluid
DAB	Diaminobenzidine tetrahydrochloride
DBD	DNA-binding domain
E3G	Oestrone-3-glucuronide
EC	Emergency contraception
ER	Oestrogen receptor
FSH	Follicular Stimulating Hormone
GnRH	Gonadotrophin releasing hormone
HRE	Hormone regulatory elements
hsp	Heat shock proteins
LBD	Ligand-binding domain
LH	Luteinizing Hormone
LNG	Levonorgestrel
MMPs	Matrix Metaloproteinases
MUC –1	Mucin glycoprotein 1
NET	Norethisterone
P	Progesterone
PBS	Phosphate buffered saline
PBST	Phosphate Buffered Saline with Tween
PC	Pill counts
PG	Prostaglandins
PGDH	Prostaglandin 15-Dehydrogenase

PLA_2	Phospholipase A_2
PR	Progesterone receptor
PR_A	Progesterone receptor type A
PR_B	Progesterone receptor type B
PRKO	Progesterone receptor knock-out
RIA	Radio-immuno assay
RT	Room temperature
SD	Standard deviation
SHBG	Sex hormone binding globulin
SR	Self report
StAR	Steroido-genic acute regulatory protein
TAF	Transactivation function
UN	United Nations
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

The world population reached 6 billion on 12th October 1999, according to the best guess of demographers. It has doubled in less than forty years, and if the decline in fertility rates falters, could yet double during our lifetime (United Nations (UN) population division 1999). The changes that occurred during the last century including the early menarche and age of first intercourse, late marriage, with many women choosing not to have children (mirrored by the highly significant decline in total fertility rate per woman (UN 1999a)), mean that the women of today are burdened more than ever "from the tyranny of excessive fertility" (Baird 1965). In order to avoid an unwanted pregnancy, sexually active women may have to adhere with a contraceptive regimen all their reproductive life – i.e. over a 40-year period. World Health Organization (WHO) reports a significant change in the use of contraception in the world, but we are still failing to meet the demand of millions of couples who want to plan their families and prevent pregnancies. In 1994, it was estimated that approximately 300 million couples in the world were using a method of contraception either that they are dissatisfied with or is unacceptable to them (UN population division 1994). Fuelled by women's increased education and awareness of the need for contraception, the customer base for contraception is likely to expand significantly over the next decade (UN 1995). Since we are in the era of the largest cohort of reproductive aged population in history, consequences of even a small difference in unwanted fertility will be catastrophic. Although the steroid hormonal regimens dominated the female methods of reversible contraceptives over the last 40 years, side effects have severely affected their acceptability. This provides the incentive for the pursuit of novel alternative 'non-hormonal' methods of contraception.

Progesterone controls most, if not all, of the key events surrounding the complex regulation of normal female reproductive function. Thus, a substance with anti-progesterone properties should be versatile not only as a contra-gestive but also as a contraceptive. Mifepristone binds with high affinity for progesterone receptor and

antagonises the actions of progesterone at its receptor level (Baulieu 1989a). It has been successfully used to induce medical termination of pregnancy in the UK, France, Sweden and in China. *Our study described herein explored the feasibility of using mifepristone as a once-a-month contraceptive pill (Chapter 3).*

Widespread use of effective emergency contraceptives (EC) is estimated to prevent millions of abortions in the world (Glasier & Baird 1997 Review). The synthetic progestin Levonorgestrel (LNG) was licensed in the UK early in the millennium, as a new EC agent. It has already been used for this purpose in several other countries. Despite this, the mechanism by which LNG acts when given in this manner is unknown. *In order to explain the post-coital contraceptive mechanism we investigated the effect of LNG on the menstrual cycle (Chapter 2).*

Non-adherence to a contraceptive method interferes with its efficacy and disrupts the evaluation of results in a research setting (Rudd *et al.* 1991; Urquhart 1991). Although a problem of great importance, the realities of patient non-compliance with contraceptives are still poorly understood. The literature on non-compliance with the use of different contraceptive methods is limited. If at all, very little progress is made in either accurately detecting, or predicting non-compliance. *We sought to obtain insight into the adherence behaviour of women taking part in a contraceptive trial assessing the feasibility of administering once a month mifepristone (Chapter 4).*

The frequent and regular laboratory assays of steroid hormones in either blood or in urine had traditionally played a major role in determining the fertile period of the cycle. In contraceptive research these assays have been used as the standard method of timing the ovulation. This approach is not only inconvenient for the volunteers but it is also expensive and time consuming to collect samples for daily assays. *We analysed the practical aspects of using a microelectronic personal fertility monitor as an alternative*

to the laboratory based hormone assays, in a study that investigated the mechanism of action of an emergency contraceptive (Chapter 5).

Withdrawal of progesterone is the physiological trigger for menstruation. In 1988, Johannisson *et al.* suggested that the menstrual bleeding which follows the administration of mifepristone in luteal phase might be due to a direct effect on the capillary vessels of the endometrium. Further understanding of the mechanism by which mifepristone induces endometrial shedding may contribute in the future to the development of novel methods of contraceptives. *To investigate this issue further, we evaluated the effects of mid-luteal phase administration of mifepriistone on endometrial parameters (Chapter 6).*

1.2 Progesterone

Progesterone is a natural steroid hormone that is produced by the corpus luteum in mammals. It is fundamental for the initiation, establishment and maintenance of pregnancy, whereas the cessation of progesterone secretion results in parturition, lactation and menstruation. Although the primary target tissues for the actions of progesterone are in the female reproductive tract, it also acts on the mammary gland (lobular-alveolar development in preparation for milk secretion and suppression of milk protein synthesis prior to parturition), on the central nervous system (mediating sexual behaviour in rodents) (Mani *et al.* 1994 a&b) and, on the thymus (inducing thymic involution) (Tibbetts *et al.* 1999). Corner and Allen were the first to isolate progesterone from the pig corpus luteum. In 1929, they demonstrated that when injected to overiectomised animals, progesterone can induce endometrial morphological changes similar to those of the early pregnancy, and has an anti-abortifacient effect (Corner & Allen 1929). Subsequently, in 1934, several independently working groups reported isolation of the crystallised progesterone (Allen & Wintersteiner 1934; Butenandt *et al.* 1934; Hartmann & Wettstein 1934). In the following year, Butenandt succeeded in

synthesising progesterone using cholesterol, and by 1938 several other synthetic progestins were commercially available.

Since then, several other important discoveries have contributed to our current understanding of this important hormone. These include the radio-immuno assaying techniques that accurately quantify progesterone by Lieberman *et al.* (1959), the identification of the uterine progesterone receptor by Milgrom & Baulieu (1970), the demonstration of the role of progesterone in the establishment of and maintenance of pregnancy by Csapo & Pulkkinen, (1978) and, the confirmation of this pivotal role of progesterone in fertility with the progesterone receptor knock-out mouse (PRKO) by Coneelly *et al.* in 2001. The pathways of progesterone action in target tissues are still not fully understood and, in many regards, distinctions remain to be made between the direct effects of progesterone and indirect consequences of progesterone regulation. Furthermore, progesterone mediates its effects through the progesterone receptor (PR) and PR is induced by oestrogen in most target tissues. Therefore, mapping out the progesterone specific effects, as distinct from those of oestrogen, is a complicated task.

1.2.1 Structure and metabolism

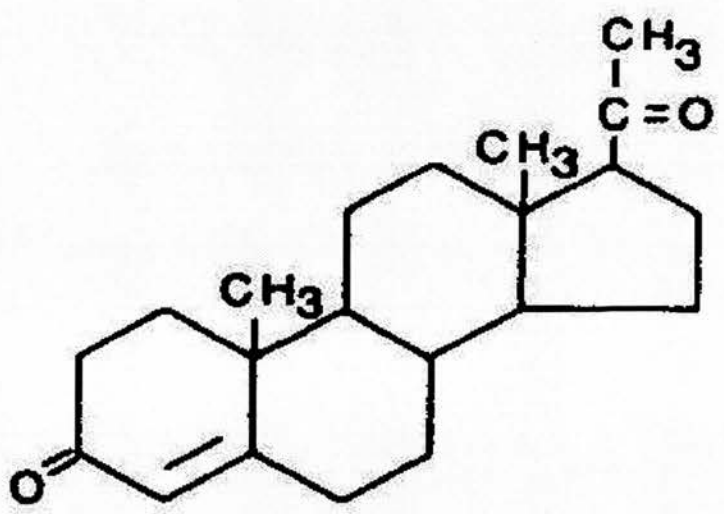


Figure 1.1 Progesterone

Progesterone is a C21 steroid (Figure 1.1) that belongs to the progestin class of ovarian hormones on the basis of its biological function. It is converted from cholesterol in the follicular cells. The delivery of cholesterol to the inner mitochondrial membrane is the rate-limiting step in progesterone synthesis. In corpus luteum, the steroido-genic acute regulatory protein (StAR) mediates this process (Review by Strauss 2000 references therein). Cytochrome P-450 enzyme system that is responsible for catalysing the cleavage of C-20, 22 bond is located in the inner mitochondrial membranes and pregnenelone (C22) is formed as illustrated in Figure 1.2 (Farkash *et al.* 1986; Lieberman & Lin 2001). Thereafter, the 3β-hydroxysteroid dehydrogenase: 5-4 isomerase enzyme complex functions as a single entity to further convert pregnanelone to progesterone. Finally, the principle catabolite of progesterone, pregnanediol, is formed in the liver (Swart *et al.* 1993) and is eliminated by the kidney.

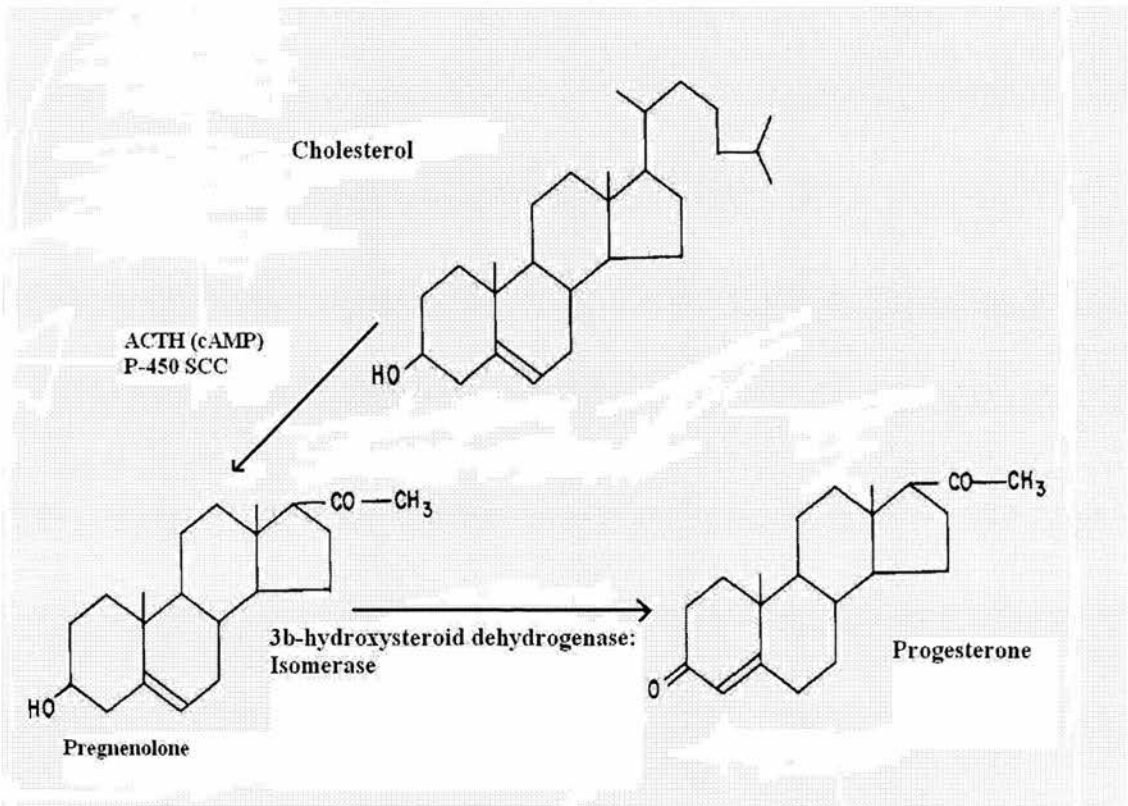


Figure 1.2 Progesterone synthesis from cholesterol

1.2.2 Progesterone Receptor (PR)

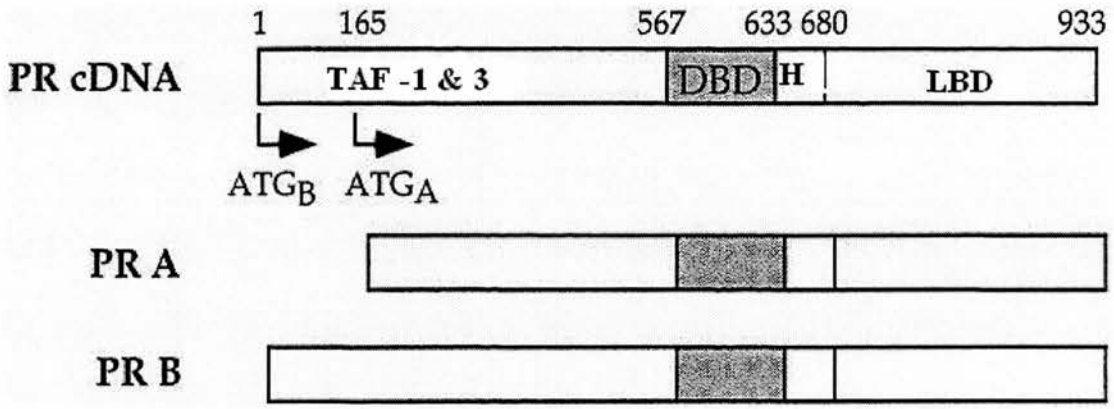


Figure 1.3 The structural organization of the Progesterone Receptor

This figure illustrates the human PR cDNA, PR_A and PR_B proteins.

TAF-1 & 3: Amino terminal containing a constitutional ligand-independent trans-activation function (TAF-1 and TAF-3); DBD: central DNA-binding domain; H: the hinge region; LBD: the ligand-binding domain. The arrows indicate the translation start site for each receptor subtype. PR_B is 933 amino acids in length and PR_A lacks the N-terminal 164 amino acids.

The gestation-promoting properties of progesterone are exerted via interacting with specific nuclear receptor proteins that are induced by oestrogen. Conversely, in most target tissues, the expression of PR is decreased by its own ligand, progesterone. Progesterone displays lower affinity to PR, when compared with oestrogen binding to oestrogen receptor (ER). At high concentrations glucocorticoids can bind to PR (Walters & Clark 1977), whilst progesterone too can bind to both the glucocorticoid receptor (GR) and the androgen receptor (AR) at similar high concentrations (Lippman *et al.* 1977; Rousseau *et al.* 1973). Universally, the cellular uptake of progesterone appears to have no impediments thus, is rapid. The retention of the hormone in target cells seems to be attributable to the presence of PR (Clark & Sutherland 1979).

1.2.2.1 Structure of the Progesterone Receptor

PR belongs to a large steroid receptor super family of ligand-activated nuclear transcription regulators. They are characterised by organisation into specific functional domains that are relatively conserved between species and family members. The "consensus" PR consists of 4 different domains (Kumar *et al.* 1987; Evans 1988; Carson-Jurica *et al.* 1990; Figure 1.3).

1. The N-terminal domain contains a constitutional ligand-independent transactivation function (TAF-1 and TAF-3), which specifies the promoter of the target gene activation (Tora *et al.* 1988; Sartorius *et al.* 1994). This region appears to differ between species (Conneeley & Lydon, 2000a).
2. The highly conserved central DNA-binding domain (DBD) interacts with specific DNA sequences -the hormone regulatory elements (HRE) - in the target genes and it also contributes to receptor dimerisation (Freedman *et al.* 1992; Peterson *et al.* 2000).
3. Sandwiched between the DBD and the ligand-binding domain (LBD) is the hinge region.
4. The LBD at the carboxy-terminal contains a second ligand-dependent transactivation function (TAF-2). In addition to the obvious ligand binding activity, this region is important for receptor dimerization, interaction with heat shock proteins (hsp), and inter- and intra-molecular silencing (Webster *et al.* 1988; Fawell *et al.* 1990; Vegeto *et al.* 1992; Lanz & Rusconi 1994; Conneeley *et al.* 2000b).

Since the discovery of PR (Laumas & Farooq 1966; O'Malley *et al.* 1969; Sherman *et al.* 1970; Milgrom & Baulieu 1970), two natural forms of the human PR (PR_A and PR_B) have been characterised, and extensively studied. Both iso-forms bind progesterone and, are derived from transcripts initiated from two distinct promotors within the same PR gene (Kastner *et al.* 1990). Their functional properties differ in a cell type, promotor, or ligand specific manner (Tora *et al.* 1988; Chalbos *et al.* 1994). Thus, existences of these

PR isoforms may add to the complexity of progesterone action. Furthermore, the tissue distribution of these PR isoforms, and their ratio in reproductive tissues is influenced by developmental status (Shyamala *et al.* 1990), hormonal status (Duffy *et al.* 1997; Mangal *et al.* 1997) and malignant transformations (Graham *et al.* 1996). The full length PR_B (114 kDa), contains an additional N-terminal transactivation region, hence in the presence of progesterone, has a greater transcriptional efficacy than the truncated PR_A version. When expressed in the same cell in equi-molar ratios, with progesterone binding PR_A & PR_B may dimerise and bind to DNA as PR_A :PR_A, PR_A :PR_B, or PR_B : PR_B (Conneely *et al.* 2001). Although usually the progesterone activated PR_B homo-dimers bind positively acting PREs to induce gene transcription, activated PR_A homo and hetero dimers appear to repress transcription of specific genes. PR_A (94 kDa) exhibit a trans-dominant negative activity, towards other steroid receptors including PR_B, ER, GR and AR (Vegeto *et al.* 1993; McDonnell *et al.* 1994 a&b; Saatcioglu *et al.* 1994). Since PR_A can act as a dominant repressor of PR_B (Vegeto *et al.* 1993; Saatcioglu *et al.* 1994), the ratio of PR_A to PR_B expression may determine the progestin responsiveness in a particular cell type.

Production of PR subtype knock-out mice by Conneely *et al.* has provided the opportunity to test their functional roles at least in the rodent model (Conneely *et al* 2001). The PR null mouse (PR_A & PR_B knock-out) showed several reproductive abnormalities including failure of ovulation, uterine hyperplasia and inflammation, defective implantation, lack of sexual behaviour in response to progesterone, abnormal morphology of the mammary gland and defective thymic immuno-adaptation to pregnancy (Lydon *et al.* 1995). The suppressive function for P in uterus appears to be due to PR_A while both the isoforms are involved in ovulatory process (Conneely *et al.* 2001). The epithelial hyperplasia seen in PR_A knockout mice may suggest that PR_B facilitates endometrial hyperplasia while PR_A inhibits it, and this may have obvious relevance to pharmacological management of endometrial dysplasias.

1.2.2.2 Progesterone binding and cellular mechanism of action of the progesterone receptor

Free PR forms an inactive, non-DNA-binding hetero-oligomeric 8S complex, which upon agonist binding dissociates in to homo-dimers with DNA binding capacity. 42 C-terminal amino acids of the LBD region are mandatory for progesterone (agonist) binding (Carson-Jurica *et al.* 1990; Vegeto *et al.* 1992). The 8S complex includes receptor-associated proteins such as heat shock protein hsp90 and immunophilin FKBP59/HBI. These proteins play a role in stabilising the hetero-oligomeric receptor complex (Baulieu *et al.* 1989 a, b&c; Lebeau *et al.* 1993). Furthermore, the multipoint hsp90 binding to PR involves both LBD and DBD thus, obliterates the DNA binding to the DBD (Bourgeois *et al.* 1984; Baulieu & Catelli 1989b). Progesterone binding induces *trans*-conformation (Allan *et al.* 1992) that initiates dissociation of the heat-shock proteins from the non-DNA binding receptor complex, homo-dimerisation (Tsai *et al.* 1988), increased receptor phosporylation (Edwards *et al.* 1993; Weigle *et al.* 1993 & 1994) thus activating the receptor.

1.2.2.3 Mechanisms of action of progesterone through classic hormone regulatory element

The activated PR binds to the progesterone regulatory element (PRE) of a target gene, a specific DNA sequence that is located in the promoter, upstream of the coding sequence. Consensus HREs consist of palindromic hexanucleotide motifs separated by three-nucleotide spacers (Beato *et al.* 1989 & 1996). Binding of the progesterone-PR complex to the two halves of the same palindromic PRE results in gene transcription via the action of RNA polymarase. How exactly the activated PR couples with the general transcriptional machinery is not well understood. Nevertheless, recent data on PR and other steroid receptors suggest a possible involvement of specific co-activators, co-repressors, specific-DNA binding transcription factors and, general transcriptional factors. It has been shown that co-activators (e.g. SRC family, CBP/p300, E3 ubiquitin-protein ligases, L7/SPA, HMG-1/2 and, SRA) are recruited by the progesterone-bound

PR and, they enhance receptor-dependent trans-activation (Rowan 2000 & references therein). Agonist-bound PR may also induce remodelling of the chromatin structure thereby facilitating the DBD and PRE interaction and recruiting transcription factors (Truss *et al.* 1993 & 1994).

The two transcription activation function areas (in the DNA binding domain & in the LBD) within the PR further regulate the initiation of gene transcription (Cadepond *et al.* 1997). Activity of TAF-1 depends on receptor and promoter types and cell-specific factors (Meyer *et al.* 1990; Vegeto *et al.* 1993), while only agonists are able to activate the TAF-2 in LBD, possibly by opposing an inhibitory activity that is located at the C-terminus of the PR (Vegeto *et al.* 1992). In addition, upon DNA binding PR undergoes N-terminal mediated changes outside the DBD (Bain *et al.* 2000).

PR, in parallel with the other members of the steroid receptor super family, is a phospho-protein and, agonist induced phosphorylation of the PR is associated with enhanced specific-DNA binding which may consequently enhance the transcriptional activity (Beck *et al.* 1992; Edwards *et al.* 1993; Weigel *et al.* 1993 & 1994).

In addition to the above described progesterone induced transcription activation, recent data has proposed alternative ligand independent pathways of PR activation, which involve altered cellular conditions due to increased intracellular kinase activity, or stimulating intracellular phosphorylation (Conneely *et al.* 2000 and references therein).

Therefore, following progesterone binding, the PR undergoes a conformational change and initiates a number of progesterone-dependent transcription processes that mediate the genomic response to the hormone.

1.2.2.4 Novel mechanisms of action through PR interaction with alternative sequence-specific transcription factors.

Interestingly, it has been shown that progesterone-PR complex is able to either repress or enhance transcription of different proteins that depended on the same transcription factor. In normal mammary epithelial cells, under the same cellular conditions but different promotor context, PR is able either repress (β-casein) or enhance (3β-HSD) Stat5 (signal transducer and activator of transcription)-mediated gene transcription. The 3β-HSD promotor region only contains a Stat5 response element and, progesterone induced 3β-HSD gene transcription appears to be Stat5 dependent. In the same cell type, the liganded PR suppressed the Stat5 dependent β-casein transcription. It has been proposed that both PR and Stat5 compete for a composite binding site in the β-casein promotor, and that occupancy by the liganded PR prevents Stat5 transactivation function (Edwards *et al.* 2000).

In breast cancer cells, PR activates the c-Src tyrosine kinase in a rapid, progesterone dependent manner and this action is independent of gene transcription but involves the prolin-rich motif of the N terminus of PR (Edwards 2002).

1.2.2.5 Non-genomic actions of progesterone

There is now compelling evidence for the existence of progesterone effects that cannot be explained by the classic model of steroid-target cell interaction. These effects tend to be incompatible with mRNA and protein synthesis (rapid and can be observed in cells that do not have trancriptional ability e.g. spermatozoa; and transcription inhibitors are unable to block these effects) and, may not involve PR or progesterone entering the cell, yet are highly specific and may involve surface membrane binding sites (Revelli *et al.* 1998). Data exist on non-genomic actions of progesterone in granulosa cells (Machelon *et al.* 1996), in amphibian oocyte maturation (Sadler *et al.* 1985; Revelli *et al.*1998 and references therein), and in spermatozoa (Bray 1999). However, the importance of these

non-genomic responses to progesterone in human female reproduction remains to be determined.

1.2.3 Endometrial steroid receptors

Immuno-histochemical studies employing monoclonal antibodies have made it possible to demonstrate the nuclear location of the steroid receptors in different endometrial compartments (McCarty *et al.* 1985; Press *et al.* 1988). During the proliferative phase, PR concentration increases in both epithelial and stromal compartments. Although, this remains high in the early luteal phase (predominantly in the glands), there is a decline in the mid- to late secretory phase (Garcia *et al.* 1988; Lessey *et al.* 1988; Berthois *et al.* 1991). The observed decrease in the PR expression during the latter part of the cycle is pronounced in the glandular epithelium, when compared to the stromal compartment (Bergeron *et al.* 1988; Lessey *et al.* 1988; Press *et al.* 1988; Snijders *et al.* 1992; Fung *et al.* 1994; Moutsatsou *et al.* 1997). Furthermore, within the stromal compartment, PR showed a specific localisation in the perivascular regions (Critchley *et al.* 1998a). Several groups have shown that in the human endometrium, the PR expression varies with the menstrual cycle phase, and reach a maximum during the early luteal phase (Janne *et al.* 1975; Bergeron *et al.* 1988; Garcia *et al.* 1988; Lessy *et al.* 1988; Press *et al.* 1988; Snijders *et al.* 1992; Fung *et al.* 1994; Zeimet *et al.* 1994; Moutsatsou *et al.* 1997). Oestrogen and progesterone sequentially regulate endometrial PR expression. Oestrogen induces PR protein synthesis by mediating an increase in PR mRNA throughout the proliferative phase (Clarke 1990) while progesterone down regulates PR at transcriptional and posttranscriptional levels throughout the secretory phase (Chauchereau *et al.* 1992). Although the smaller A-receptor remains the phenotypically dominant iso-form in the endometrium, the PR_A: PR_B expression ratio is increased in the peri-menstrual period of the cycle (Tung *et al.* 1993; Mangal *et al.* 1997). The transdominant negative effect of PR_A may produce the observed inhibitory effects of progesterone and antiprogesterones on ER expression. Furthermore, in mid-luteal phase endometrial glands, PR_B may be the predominant PR subtype (Mote *et al.* 1999).

In the secretory endometrium, AR expression had been observed predominantly in the stroma with barely detectable AR immunoreactivity in the glandular epithelium (Slayden *et al.* 2001). Immediately before menstruation in the late secretory phase, a decrease in stromal AR immuno-staining had been reported (Slayden *et al.* 2001). In contrast, an up-regulation was observed in glands as well as in stroma, following the early luteal phase administration of mifepristone (Slayden *et al.* 2001).

1.2.4 Myometrial PR

Recent evidence suggests that the non-pregnant uterus acts as a peristaltic pump during the menstrual cycle with directed sperm transport being one of the main functions (Lyons *et al.* 1991; Kunz *et al.* 1996; Leyendecker *et al.* 1996). Noe and colleagues demonstrated a cyclical pattern of ER and PR expression in the subepithelial myometrial cells, which parallels that of the endometrium. They went on to suggest that progesterone might be involved in the very early processes of reproduction, by regulating the cyclical uterine peristalsis (which is confined to the endometrium and sub-endometrial myometrium) that assists rapid and sustained sperm transport, in addition to the action on the endometrium. The outer myometrial layers did not exhibit a cyclical pattern of PR expression, but the immuno staining remained strong throughout the whole cycle. This cell-specific differential receptor regulation might be functionally significant in suppressing the action of progesterone in the subendometrial myometrium (supressing the cyclical uterine peristalsis) in the presence of high circulating concentrations of this hormone required for continuing action in the outer myometrial layers (maintaining myofibrils at a quiescent state) (Noe *et al.* 1999).

1.2.5 Progesterone effects on the uterus

Progesterone acts upon the endometrium to initiate either directly or indirectly appropriate changes in structure responsible for the required functional end points, implantation, regeneration and repair. If fertilisation were to occur, the high circulating levels of progesterone that result, facilitate implantation, stimulate uterine growth and

oppose the action of factors involved in myometrial contractility. Conversely, at the end of an infertile cycle, withdrawal of progesterone will induce menstruation. Moreover, the anti-proliferative / anti-oestrogenic effects of progesterone in the endometrium have been extensively studied; They include the down-regulation of ER (Hsueh *et al.* 1970; Katzenellenbogen *et al.* 1980), the increased metabolism of oestradiol to less active oestrone (Tseng & Gurpide 1975), and the decrease of oestrogen-induced proteins and proto-oncogenes (Bhakoo *et al.* 1977; Kirkland *et al.* 1992; Elger *et al.* 2000).

1.3 Levonorgestrel

LNG (systemic name 17β hydroxy- 17α ethinyl- 13β ethyl- 4 gonen- 3 – one) is the biologically active dextro-rotatory isomer of the synthetic progestin norgestrel, and has more potent progestational and anti-ovulatory action than the naturally occurring progesterone (Khan *et al.* 1983; Stanczyk *et al.* 1994). Various formulations of LNG are currently being used clinically, including; (i) in combination with ethinyloestradiol for the combined oral contraceptive pill or for Yuzpe regimen of post coital contraceptive, (ii) in combination with 'natural' oestrogens for hormone replacement therapy, (iii) on its own as LNG only post coital contraception and as LNG releasing IUCD, intravaginal rings, and subcutaneous implants. Pharmacokinetics of LNG depends on the particular formulation.

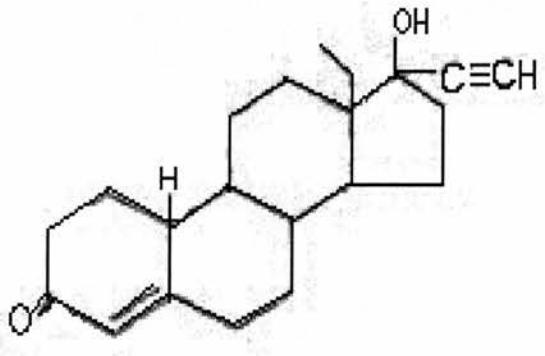


Figure 1.4 Structure of Levonorgestrel

1.3.1. Pharmacokinetics of LNG

Since LNG is not metabolised through the entero-hepatic circulation (Fotherby *et al.* 1994), the bio-availability of the oral dose is high. When administered orally, it is rapidly absorbed, and achieves the maximum serum concentration (t max) within 2 hours (Goebelsman *et al.* 1986; He *et al.* 1990; Kuhnz *et al.* 1992; Kook *et al.* 2002). In common with the other synthetic gestogens (Fotherby 1983) LNG shows a large inter- and intra individual variability in the terminal half-life of elimination (mean values ranging from 8 - 26 hours) (Weiner *et al.* 1976; Goebelsman *et al.* 1986; Back *et al.* 1987; Fotherby *et al.* 1990; Tremblay *et al.* 2001; Kook *et al.* 2002). The importance of hormone sensitive sex hormone binding globulin (SHBG) in LNG pharmacokinetics has been reported by several investigators (Victor *et al.* 1976 &1977; Affandi *et al.* 1987). In blood, LNG binds to both albumin, and (with high affinity) to SHBG. LNG dissociates slowly from the stable LNG-SHBG complex (Juchem *et al.* 1990; Fotherby *et al.* 1994), and only the unbound fraction in serum (less than 2% of the total drug) is responsible for the biological action. On the other hand, LNG directly affects serum SHBG, and a single dose of 0.75 mg LNG was able to reduce the circulating SHBG by 25% (He 1990). However, this action is reversed by the concomitantly administered ethinyloestradiol, thereby affecting the pharmacokinetics of LNG (Fotherby *et al.* 1990). The studies evaluating the pharmacokinetics of LNG had small numbers, and did not determine the SHBG level at the time of administration of LNG. These deficiencies may explain the variability seen in the pharmacokinetic profile of LNG since the range of SHBG levels among healthy women is reported to be wide (Fotherby *et al.* 1995). The metabolites of LNG are present in the circulation at greater concentrations than LNG for a longer time period (Warren *et al.* 1974). If biologically active, they may sustain the progestagenic effects for a much longer period than expected. Once more, this possibility is yet to be elucidated. Therefore, the complex pharmacokinetics of LNG makes its correlation to the pharmacodynamic activity difficult. However, recent studies have shown that following 0.75mg of LNG taken twice 24 hours apart, the plasma LNG level remains above 1 nmol/L for approximately 4.5 days (Tremberly *et al.* 2001). This is of particular

importance, as it is the minimal plasma level of LNG required to inhibit ovulation (Nilsson *et al.*1980).

1.4 Mifepristone

Agents that antagonise the effects of progesterone were actively sought even prior to the present day understanding of the cellular mechanism of action of hormones. Although unsuccessful in finding a synthetic anti-progestagen, Pincus was the first to postulate the potential value of a compound with anti-gestagenic activity in fertility control (Pincus 1965). Since then, many others have made several important contributions (Sherman *et al.* 1970; Belanger *et al.* 1981) that finally led to the discovery of mifepristone (RU 38486, subsequently abbreviated to RU 486), which was the first potent anti-gestagen to be synthesised (Philibert *et al.* 1981). Two decades have passed since and, unfortunately the political controversy surrounding mifepristone as an "abortion pill" has hindered its many other potential therapeutic uses being put to practise.

Mifepristone exerts its potent antiprogesterone and antiglucocorticoid action in humans by binding to the intracellular receptors of the antagonised hormones. Despite the close structural relationship between the steroid receptors, mifepristone does not bind to ER or the mineralocorticoid receptor (MR) yet demonstrates high affinity for PR, GR, and also (somewhat lower affinity) for AR. Upon binding mifepristone maintains the PR in a biologically inert conformation that precludes progesterone binding (Evans *et al.* 1988; Baulieu 1989) and itself does not activates the PR.

1.4.1. Structure of mifepristone

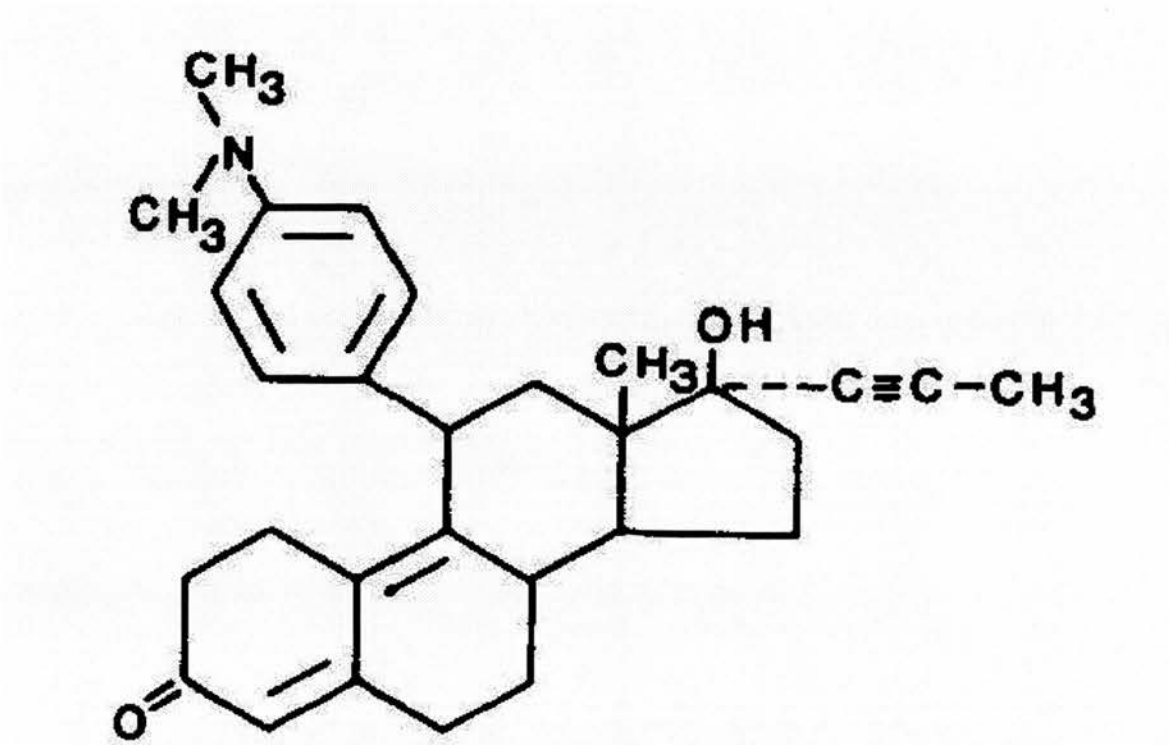


Figure 1.5 Mifepristone

Mifepristone is a 19-norsteroid that lacks the C19-methyl group of natural progesterone and of glucocorticoides. The chemical structure of mifepristone shows two important distinctive differences from the steroidal skeleton, a 4-dimethyl amino-phenyl group substituted at the 11β position, and a 1-propynyl-chain substitution at the 17α position (Figure 1.5). The higher binding affinity of mifepristone to PR is thought to be due to both these variations, while the size and positioning of the former determines the anti-progesterone action (Evans *et al.* 1988; Baulieu 1989; Garcia *et al.* 1992; Spitz *et al.* 1993). The17α-propynyl side chain appears to confer pure-glucocorticoid activity, and various modifications at C-17 position of the RU 486 structure resulted in compounds with relatively lower anti-glucocorticoid activity. However, to date no pure progesterone antagonist that is totally devoid of glucocorticoid activity has been described.

1.4.2 Pharmacokinetics of mifepristone

Mifepristone is usually administered orally as a single or multiple doses and the dosage varies from 0.5 mg to 800 mg daily in various studies. The oral dose is absorbed rapidly (70%) and is subject to the first-pass metabolism in the liver. Bio-availability after the oral dose in humans (40%) does not appear to be affected by the pregnant status or the ethnicity but it is reduced in some animal species (Spitz *et al.* 1993, Sarkar 2002). Mifepristone is metabolised in the liver and is excreted mainly in urine and faeces as biologically active demethylated and hydroxylated metabolites.

1.4.2.1 Receptor binding and ligand-induced trans-conformation

Mifepristone is a potent antagonist of progesterone action in vivo, however the mechanism of this antagonism is poorly understood. Mifepristone binds to PR with a relative binding capacity five times that of progesterone (K of dissociation <1 nM) and does not activate the receptor (Philibert 1984; Skafar *et al.* 1991). The affinity of mifepristone for the GR is also of same order of magnitude as that for PR. The magnitude of the anti-glucocorticoid activity however, is influenced by the phase of cicardian rhythm and the negative pituitary feed-back of cortisol secretion and, requires a higher dose than that which has anti-progesterone activity (Healy *et al.* 1983 & 1985; Gaillard *et al.* 1984; Bertagna *et al.* 1984).

The PR of most species including humans contains a large hydrophobic pocket that can accommodate the equally hydrophobic 11β substitution of mifepristone (Teutsch *et al.* 1985). Mifepristone appears to require a set of N-terminal region amino acids of LBD on the PR that overlap with the progesterone binding, but are non-co-incidental. A monoclonal-antibody to a 14 amino acid sequence in the C-terminal of LBD only blocks progesterone binding, but not mifepristone (Weigel *et al.* 1992). Point mutations have been identified in the LBD that distinctly affect binding of either mifepristone {at Gly722 of the human PR (Benhamou *et al.* 1992)} or, progesterone {Cys575 of the chicken PR (Baulieu 1993)} but not the other; and a C-terminal mutant PR has been

shown to bind only mifepristone, but not progestin (Vegeto *et al.* 1992). The qualitative property of hormone antagonism however, does not appear to equate to mere binding ability. Although mifepristone does not bind to chicken PR (cPR), a change of a single amino acid in the N-terminal region of cPR LBD (Cys 575 to Gly) allows binding of both mifepristone and RU 39115 (RU 39115 is an antagonist of the human PR and, lacks the N-dimethyl substituent of mifepristone). Upon this binding however, the antiprogesterone activity is only observed with mifepristone. RU 39115 on the other hand, exhibits agonist activity in the mutant receptor (Gronemeyer *et al.* 1992; Baulieu 1993). Therefore, the overall structure of the LBD and the antagonist and, the TAF-2 function may influence the biological activity of a steroid hormone agonist or antagonist.

Mifepristone competes with progesterone for binding to PR and promotes many of the normal activation steps elicited by progesterone, including dissociation of the heat-shock proteins, dimerisation, and acquisition of high affinity for target DNA binding (Baulieu *et al.* 1988; El-Ashry *et al.* 1989; DeMarzo *et al.* 1992; Gass *et al.* 1992; Beck *et al.* 1993a). Although mifepristone-PR complex has higher affinity for PRE than that of progesterone-PR complex, upon binding to the PRE only the latter and not the former, activates the transcriptional machinery. Therefore, mifepristone is defined as a type II antagonist (other antiprogestagenic substances which form complexes with PR but are unable to bind to PRE (e.g. ZK 98299) are defined as type I antagonists) that appears to recapitulate the early steps of agonist-activation of receptor yet fails to activate a transcriptional response. Our understanding of the molecular mechanisms of this receptor inactivation is still far from complete. Nevertheless, the existing evidence is as follows. Mifepristone-PR complexes may form inactive, mixed ligand-dimer hetero-dimers with progesterone-PR complexes, while actively competing with them for PRE binding sites (Edwards *et al.* 2000); mifepristone induces 'an inappropriate' structural conformation at the extreme carboxy-terminus of the LBD, which is distinct from that induced by progesterone (Allan *et al.* 1992; Weigle *et al.* 1992); This altered conformation can prevent both the agonist-dependent TAF-2 activity and the binding of

PR co-activators that enhance receptor-dependent transcription such as SRC (Edwards *et al.* 1999 & 2000); Mifepristone may also promote recruitment of inappropriate co-activators that are not selected by progesterone such as L7/SPA (Jackson *et al.* 1997); Additionally, it may actively recruit PR co-repressors and the co-activator and co-repressor concentration ratios may determine the antagonist or partial-agonist activity of mifepristone (McDonnell *et al.* 1992; Jackson *et al.* 1997; Smith *et al.* 1997).

In particular tissues, mifepristone may behave as a progesterone agonist. In the absence of progesterone, mifepristone exhibits a progesterone-like anti-oestrogenic effect in an oestrogen-primed endometrium (Wolf *et al.* 1989; Van Uem *et al.* 1989). However, if progesterone was present mifepristone acts as a pure antagonist. The repressor role of PR_A on ER has been suggested as the explanation for this anti-oestrogenic effects of mifepristone (see Cadepond 1997 and references therein). Recently, Brenner *et al.* suggested that this non-competitive anti-oestragenic effect of mifepristone seen on rhesus macaque endometrium is mediated via inducing AR expression (Brenner *et al.* 2002). Similar to progesterone, mifepristone inhibits prolactin Stat5- mediated induction of β-casein Luciferase receptor in the mammary epithelial cells. It has been postulated that this is due to the occupancy of the composite DNA-binding element located in the β-casein promotor by the mifepristone-PR complex which precludes the Stat5 binding, thereby suppressing Stat5 trans-activation function (Edwards *et al.* 2000). In vitro studies have exposed conditional agonist activity similar to other type II antagonists with mifepristone. Alteration of cellular conditions by either activation of protein kinase A or stimulation of cAMP signalling pathway resulted in antagonist-to-agonist switch in mifepristone activity in vitro (Beck *et al.* 1993b; Nordeen *et al.* 1995). It is of further interest that the weak partial agonist activity is associated with only mifepristone bound PR_B but not PR_A (Leonhardt & Edwards 2002).

In summary, the structure of mifepristone and of the LBD combine ultimately to direct the trans-conformation of PR, and DNA binding; but do not activate the TAF-2 or

induce transcription and thus, mifepristone acts as an antagonist. The production of a conformational change in the PR distinct from that induced by progestins is a central property of mifepristone.

1.4.3. Toxicology

1.4.3.1 Short-term administration

A single oral dose of mifepristone is eliminated within 6-7 days of administration (Sarkar 2000). The effects of short-term administration of mifepristone have been well characterised and, appear to be safe. Administering 200 mgs of mifepristone daily for 8 days to healthy men resulted in a hormonally detectable antiglucocorticoid state and a reversible cortisol overproduction with preservation of adrenocortical and pituitary reserves. Importantly, no concurrent clinical symptoms of peripheral cortisol deprivation were observed with these effects (Bertagna *et al.* 1994). Others have shown that the disinhibition of the pituitary-adrenal axis is only observed during the morning hours of the circadian rhythm, thus lowering the dosage and changing the time of administration may be important to minimize the side effects (Gaillard *et al.* 1984). One study reported that mifepristone at high doses (10 mg/Kg taken daily for a week) induced a diffuse maculo-papular erythematous cutaneous eruption within 2 weeks of discontinuation of the drug in the majority (73%) of the healthy male volunteers. Histologically, this generalised examthum showed a diffuse dermal peri-vascular lymphocytic infiltration that resolved spontaneously (Laue *et al.* 1990). However, none of the affected subjects demonstrated an increase in the eosinophil count, which does not support the possibility of drug hypersensitivity or glucocorticoid deficiency being responsible for the skin reaction. Moreover, after taking even higher doses (20 mg/kg) for longer periods, others have not developed similar complications (Chrousos *et al.* 1988).

1.4.3.3 Chronic administration

Most novel therapeutic indications of mifepristone require long-term administration (e.g. breast cancer, endometriosis) and, its antiglucocorticoid action has raised the issue of safety with chronic exposure to the drug. Prolonged administration of daily low doses was not associated with a cumulative increase in plasma concentration of the drug (Heikinheimo *et al.* 1987 & 1989; Sarkar 2002). At doses above 200 mg a day, clearance of mifepristone from the body appears to reach a limiting value and this may produce elevated plasma levels over a long duration (Sarkar 2002). In a small proportion of volunteers, when high doses of mifepristone was administered continuously for more than several days, symptomatic glucocorticoid deficiency has been observed. Chronic exposure of 200 mgs a day was associated with symptoms of cortisol deprivation that were severe enough to be treated with concomitant prednisolone (Lamberts *et al.* 1991). Lambert *et al.* postulated that mifepristone activated and reset the hypothalamo-pituitary-adrenal axis at a higher level. However, this observation has not been reported by others, using a similar regimen for as long as 15 months for the treatment of unresectable meningiomas (Heikinheimo *et al.* 2000). In pre-menopausal women, a similar regimen induced amenorrhoea, or metrorrhagia (in one case with endometrial hyperplasia) (Grunberg *et al.* 1993). The main concern with this chronic dose however, was the reported increase in oestrogen (as a consequence of the compensatory overproduction of androgen), which might limit the use of mifepristone in treating oestrogen dependent cancer. Conversely, recent data, which demonstrated a low oestrogen level with no discernible change in SHBG after chronic 200 mg daily dose, suggest that enhanced systemic oestrogen effects are unlikely during long-term mifepristone treatment (Heikinheimo *et al.* 2000).

Studies using long-term daily doses of mifepristone up to 10 mg/kg, or using once-off doses of up to 25 mg/kg, did not report any other adverse events. Since the doses required for most potential therapeutic applications of mifepristone are well within these dose limits, the safety aspect of mifepristone appears to be reassuring.

1.4.3.2 Teratogenicity

The use of mifepristone in the induction of abortion and as a contraceptive agent and the fact that it traverses the placental barrier (Hill *et al.* 1991), raises concerns about the possibility of teratogenic effects in infants who are exposed to mifepristone during early gestation (Doherty *et al.* 1992). Unfortunately, the available data on this possibility are rather limited. In rabbits, even at sub-abortive doses mifepristone induced foetal abnormalities (Yost *et al.* 1986; Wolf *et al.* 1990). However, monkey embryos did not show teratogenicity when exposed to mifepristone either before implantation (10^{-7}M) or immediately post-implantation (50 mg/day for 7 days) (Wolf *et al.* 1990). Henrion et al in 1989 reported a single case in which administration of mifepristone in the first trimester was associated with second trimester fetal-malformation (Henrion *et al.* 1989). In a more recent report, Sitruk-Ware *et al.* reviewed 71 cases of continuing pregnancy following failed early medical termination (occurred between 1987-1998) and reported only one case of fetal abnormality after the use of mifepristone alone. There were 7 further cases of fetal abnormality following treatment of mifepristone in combination with prostaglandins (Sitruk-Ware *et al.* 1998). No other instances of possible teratogenicity had been reported despite the growing use of mifepristone in inducing medical abortion around the world. Conversely, there have been reports of human fetuses that have been exposed to mifepristone but not subsequently aborted, developing normally (Lim *et al.* 1990).

1.5 Menstrual cycle

Throughout a woman's reproductive life, the dynamic relationship between the pituitary and ovarian hormones allows the cyclical sequence of events, which commences with folliculogenesis and proceeds to extrusion of a mature oocyte and transformation of the follicular granulosa cells into a corpus luteum which eventually undergoes luteolysis, thus terminating an infertile cycle.

1.5.1 Progesterone regulation of the hypothalamo-pituitary-ovarian axis

The feed back mechanism that exists between the ovary and the hypothalamo-pituitary unit is responsible for the normal cyclical ovarian activity (Knobil *et al.* 1980). The anterior pituitary produces LH and Follicle Stimulating Hormone (FSH) in a pulsatile fashion in response to the hypothalamic neuropeptide; gonadotrophin releasing hormone (GnRH). The ovarian hormones oestrogen, progesterone and inhibin also influence this regulation, and conversely, LH and FSH directly regulate these hormones. Normal LH secretion (in a pulsatile manner) and ovulation are dependent on the release of GnRH pulses that happen at a critical frequency and amplitude (Dierschke *et al.* 1970; Leyendecker *et al.* 1979). The LH pulses increase in frequency immediately before ovulation, then following ovulation slow down while increasing their amplitude in the first half of the luteal phase (Backstrom *et al.*1982; Wildt *et al.*1981; Filicori *et al.* 1986). It has been shown that the pre-ovulatory surge of LH triggers ovulation by initiating a series of changes in the dominant follicle causing the rupture and the subsequent release of the ovum. Furthermore, LH can stimulate a transient increase in PR mRNA expression in pre-ovulatory follicular granulosa cells suggesting an LH induced progesterone responsiveness in those cells (Natraj *et al.* 1993; Park-Starge *et al.* 1994). Normal women have a pre-ovulatory rise in progesterone preceding the initiation of the mid-cycle LH surge (Hoff *et al.* 1983). This is thought to facilitate oestrogen in releasing the LH surge (Odell *et al.* 1968; Chang & Jaffe 1978; March *et al.* 1979 & 1981; Wildt *et al.* 1981; Lui & Yen 1983; Mahesh & Muldoon 1987; Permezel *et al.* 1989; Leyendecker *et al.* 1990) and may also play an important role in the contemporaneous discharge of the gonadotrophins (Aono *et al.* 1976).

Presently available evidence for the effects of progesterone on the neuro-endocrine axis during the luteal phase is questionable, since studies using antiprogesterone have not always produced the expected results (Herrman *et al.* 1982; Critchley *et al.* 1988; Garzo *et al.* 1988; Shoupe *et al.* 1990). After ovulation, the facilitatory role of progesterone in releasing LH reverses rapidly into one of an inhibitory nature, (Leyendeker *et al.* 1972;

Drouin & Labrie 1981; Araki *et al.* 1985) changing the high frequency, low amplitude LH pulses of the follicular phase to pulses of low frequency and high amplitude (Hoff *et al.* 1983; Soules *et al.* 1984). The pulse frequency of LH declines progressively during the luteal phase and correlates with cumulative progesterone exposure (Filicori *et al.* 1984; Soules *et al.* 1984). Moreover, exogenous progesterone has been shown to decrease LH pulse amplitude (Soules *et al.* 1984). Endogenous opioids appear to influence LH pulsatility via inhibiting GnRH secretion (Rasmussen *et al.* 1983). Since the maximum suppression occurs when high progesterone levels are seen (Soules *et al.* 1984; Shoupe *et al.* 1985) and this effect is reversed by naloxone infusion in the luteal phase (Quigley & Yen 1980), it was thought that progesterone was important in modulating the endogenous opioid mediated inhibitory process (Quigley *et al.* 1980; Shoupe *et al.* 1985). More complex actions of anti-gestagens that are not solely due to progesterone antagonism may explain the unexpectedly different results seen in studies using anti-progesterone (Herrman *et al.* 1982; Critchley *et al.* 1988; Garzo *et al.* 1988; Shoupe *et al.* 1990). Nevertheless, immunisation of the ewe against progesterone has consistently produced an increase in the LH pulse frequency supporting the inhibitory effect of progesterone on gonadotrophin secretion (Thomas *et al.* 1987).

1.5.2 Control of Ovarian cycle

In humans, folliculogenesis, ovulation and corpus luteum formation occur under pituitary gonadotrophic control.

1.5.2.1 Follicular phase

Various ovarian follicular compartments interact in a highly integrated manner to respond to cyclic gonadotrophic hormones by secreting sex steroids and producing a fertilizable, mature ovum. Progesterone and oestrogen levels remain low during the menses, and thereafter, in the follicular phase, the developing Graafian follicle produces increasing levels of oestradiol (Yoshinga *et al.* 1978). FSH stimulates the growth of the follicles beyond the stage of antrum formation (Baird 1987 & 1990 and references

therein). By the mid-proliferative phase of the cycle, FSH-inducible granulosa cells of the apparent single dominant follicle (≥10 mm diameter) produce increasing amounts of oestrogen by aromatising the androgens derived from LH-dependent theca cells (Yoshinga *et al.* 1978). Oestrogen and inhibin secretion from the enlarging preovulatory dominant follicle (usually one per menstrual cycle) suppresses (negative feed back) the pituitary FSH release, while inducing an LH surge, which initiates ovulatory process (Baird 1990 and references therein). Ovulation seems to occur approximately 36 hours after the onset of the LH surge (Seibel *et al.*1982).

1.5.2.2 Control of Ovulation

The LH surge initiates a cascade of proteolytic events that control ovulation and, also induce PR gene expression in granulosa cells of the mature preovulatory follicles (Natraj *et al.* 1993). Furthermore, around the time of the mid-cycle LH surge in women, pre-ovulatory follicles appear to secrete increasing levels of progesterone (Zander *et al.* 1954; Hoff *et al.* 1983; Djahanbakhch *et al.* 1984). The presence of PR in most follicular cell types, failure of ovulation in PR null mice despite having mature preovulatory follicles (Suzuki *et al.* 1994; Lydon *et al.* 1995) and, the inability of the mature follicle to rupture in the absence of mid-cycle progesterone secretion (Schenken *et al.* 1986) suggest that the ovulatory process is regulated by progesterone. It is postulated that this regulatory pathway may involve relaxin, kallikrein, proteases (e.g. cathepsin L, ADAMTS-1), and plasminogen activator (Yki-Jarvinen *et al.* 1985; Too *et al.* 1984; Tanaka *et al.* 1992; Robker *et al.* 2000).

1.5.2.3 Luteal phase

The luteal phase of the cycle is dominated by progesterone arising from the corpus luteum. Conversely, formation of corpus luteum appears to be progesterone-dependent and, indeed PR is expressed in the corpus luteum despite high local progesterone concentrations (Aladin Chandrasekher *et al.* 1994). After ovulation, progesterone levels reach a peak by day 8 or 9 and, if implantation does not occur, progesterone declines by

day 10 -12 (first gradually and then precipitously). In the latter part of the infertile cycle, the corpus luteum regresses, stops the release of progesterone and, consequently a decline is observed in the circulating levels. This withdrawal of progesterone causes the endometrial pseudo-decidua to degenerate, which results in menses. Luteal regression permits initiation of a subsequent cycle, by allowing the development of a fresh batch of follicles.

1.5.3 Effects of mifepristone on the hypothalamo-pituitary-ovarian axis and on the ovarian cycle

Mifepristone binds to PRs in the hypothalamus, the anterior pituitary and in the ovary as well as the uterus. It thereby has multiple effects on the hypothalamic-pituitary-ovarian axis and on the ovarian cycle depending on the time of the cycle and the dose used.

1.5.3.1 Follicular phase administration of mifepristone

During the early follicular phase except for a minimal decrease in oestradiol level, mifepristone (at a dose of 3 mg/kg) has no effects on the ovarian cycle (Stuenkel *et al.* 1990). However, later in the follicular phase, mifepristone arrests folliculogenesis and inhibits or delays ovulation, while decreasing oestradiol and LH levels during treatment (Shoupe *et al.* 1987; Permezel *et al.* 1989; Stuenkel *et al.* 1990). In vitro studies have shown mifepristone to inhibit LH secretion by cultured rat pituicytes (Wolf *et al.* 1989). Additionally the reduction of LH pulse amplitude seen after late follicular administration of mifepristone to women suggests a pituitary site of action (Permezel *et al.* 1989). Therefore, the inhibition of ovulation seen after administering high doses (> 50 mg) in the mid-late follicular phase of the cycle seems to be due to the suppression of the secretion of pituitary gonadotrophins (both LH and FSH) thereby arresting follicular growth (Luukkainen *et al.* 1988; Nieman 1993; Liu *et al.* 1987; Shoupe *et al.* 1987; Permezel *et al.* 1989). Afterwards, follicular recruitment can reinitiate once the treatment is discontinued and ovulation can occur approximately 2 weeks later (Liu *et al.* 1987; Shoupe *et al.* 1987).

The delay of the mid-cycle LH surge seen after continuous administration of mifepristone (1 mg daily, over 5-15 days after the dominant follicle selection) in the late follicular phase can be prevented by concomitantly administered exogenous progesterone (Batista *et al.* 1992 a). The mechanism for this appears to be via inhibition of the positive feed back effect of oestrogens (Baird *et al.* 1995). It has been shown that daily administration of 2 mg of mifepristone does not affect the pulsatile pattern of LH secretion (Baird *et al.* 1995). Therefore, the impairment of oestrogen induced LH surge is not due to an effect of mifepristone at the hypothalamic level and nor is it due to a decreased responsiveness of the pituitary to exogenous GnRH. On the basis of these observations Baird *et al.* concluded that mifepristone at this dose (2 mg daily) interferes with the mid-cycle LH surge by a complex mechanism, possibly by directly reducing the sensitivity of pituitary gonadotroph to the positive feed-back effects of oestrogen.

It is important to note that the above studies reported blocking of the LH surge when multiple doses of mifepristone were administered continuously in the follicular phase. The lowest single dose, which will prevent the LH surge and ovulation consistently, is not known and once the LH surge is initiated mifepristone does not seem to be able to arrest ovulation (Liu *et al.* 1987; Marions *et al.* 2002; Brown, A. unpublished data through personal communication).

1.5.3.2 Luteal phase administration of mifepristone

In the mid-luteal phase a single dose of mifepristone (5 mg/kg) acutely increases both the amplitude and the frequency of LH pulses (Critchley & Baird 1988). After this initial effect, some authors have reported a decline in the secretion of total LH and in the pulse amplitude, but no discernible effect was seen on the pulse frequency (Garzo *et al.* 1988; Shoupe *et al.* 1990). It has therefore been suggested that this failure of an anti-progesterone to influence LH pulse frequency may be due to a lack of a direct hypothalamic effect. Although others have reported a decrease in LH pulse frequency after mifepristone, due to the low frequency of LH pulses detected, the validity of their

result is uncertain (Schaison *et al.* 1985). Low doses, 10 mg for example, do not affect LH levels (Greene *et al.* 1992). Therefore, during the mid-luteal phase, mifepristone may initially relieve a suppressive effect of progesterone on the frequency and amplitude of LH pulses, and thereafter it may have a direct pituitary action in decreasing the LH pulse amplitude. Moreover, naloxone did not reverse this mifepristone (a single dose of 100 mg mifepristone) induced decrease in the LH pulse amplitude (Shoupe *et al.* 1990) suggesting that the inhibitory hypothalamic effect of endogenous opioid peptides may not be fully dependent on progesterone as previously thought.

In the late luteal phase a single dose of 600 mg of mifepristone significantly reduces both the frequency and amplitude of LH pulses, suggesting a hypothalamic-pituitary action (Garzo *et al.* 1988).

In summary, mifepristone acts at multiple sites within the hypothalamic-pituitary-ovarian axis to produce the observed effects.

1.6 Endometrium and Menstruation

Endometrium is a specialised organ that is composed of two layers: a superficial functional layer and a basal layer. During the reproductive age, the superficial layer undergoes cyclical changes closely controlled by the ovarian hormones, and disappears after the menopause. It is composed of epithelial cells that line the uterine cavity, simple and tortuous glands, and stroma with cells of different lineages and vessels. Following menstrual shedding at the end of a cycle, the functional layer regenerates from the reserve cell layers in the basal zone.

Before ovulation, the oestrogen dominated proliferative phase of the endometrium is characterised by glandular and stromal mitoses. Progesterone, which is the dominant hormone in the post-ovulatory secretory endometrium, inhibits proliferation while

inducing the synthesis and the release of endometrial secretory products and neovascularisation (Critchley & Healy 1998b and references therein).

Therefore, the menstrual cycle can be defined as a repeated expression of the action of the hypothalamo-pituitary-ovarian system with associated structural and functional changes in the target tissues of the reproductive tract (Yen *et al.* 1991).

1.6.1 Endometrial vessels

Sex steroids have shown to have a direct effect on the vessels in the upper functionalis layer of the endometrium (Schmidt-Matthiesen *et al.* 1963; Johannisson *et al.* 1986) and these vessels may play an important role in implantation (Akerlund 1991). Following the original report by Markee in 1940, the increasing need for efficient contraceptive methods and improvement of technology related to assisted conception have renewed interest in studies of the human endometrium, including endometrial vascularisation. The rate and the volume of the blood flow to the superficial layers of the endometrium are thought to be regulated via the dynamic changes of these vessels (Ramsey 1977; Peek *et al.* 1992). During the pre-ovulatory period, the capillaries have a narrow lumen and are thin-walled. The discrepancy in growth rate (the five fold increase in the length of the spiral arteries while the endometrium thickness only doubling) that is apparent during the proliferative phase might be the reason for the pre-ovulatory coiling of these arteries (Markee 1940). Ovulation seems to provoke an immediate dilatation of the endometrial capillaries (Peek 1992). Herein after, in the luteal phase of the cycle, the vessels in the sub epithelial capillary plexus sustain this dilatation (up to day LH +7/ +9) up to the time of implantation (Bartelmez 1956; Sheppard *et al.* 1980; Seppala *et al.* 1991). Furthermore, this dilatation of vessels correlates with the serum progesterone levels circulating in the plasma 72 hours preceding biopsy and coincides with the development of stromal odema (Peek *et al.* 1992). Later, immediately preceding the onset of menses, the endothelial cells undergo contraction and degeneration (Peek *et al.* 1992).

1.6.2 Menstruation

The superficial or functionalis layer of the endometrium disintegrates at the end of an 'unsuccessful' cycle where conception has not occurred, and this process is defined as menstruation. Menses is also the climax of events that occur in the cells and vessels of the endometrium, which follow the natural withdrawal of progesterone. The current understanding of the mechanism of menstruation stems from a series of outstanding experiments performed by Markee in 1940. Using endometrial auto-transplants in the anterior chamber of the eye of rhesus monkeys, he produced a detailed account of vascular changes associated with menstrual bleeding. Subsequently two hypotheses have being advocated as the trigger mechanism for menstruation: the inflammatory hypothesis focused on pro-inflammatory cells, MMPs, chemokines and cytokines (Finn 1986; Salamonsen *et al.* 1999 and references therein), while the vasoconstrictor hypothesis emphasised the actions on prostaglandins (Baird 1996) and endothelins (Marsh 1996; Campbell & Cameron 1998). The vasoconstrictor hypothesis seems more plausible and thus, is progressively gaining recognition (Baird 1996, Figure 1.6; Critchley *et al.* 2001). Moreover, this second hypothesis integrates the previously suggested inflammatory pathway at a downstream level to the initial step, vasoconstriction.

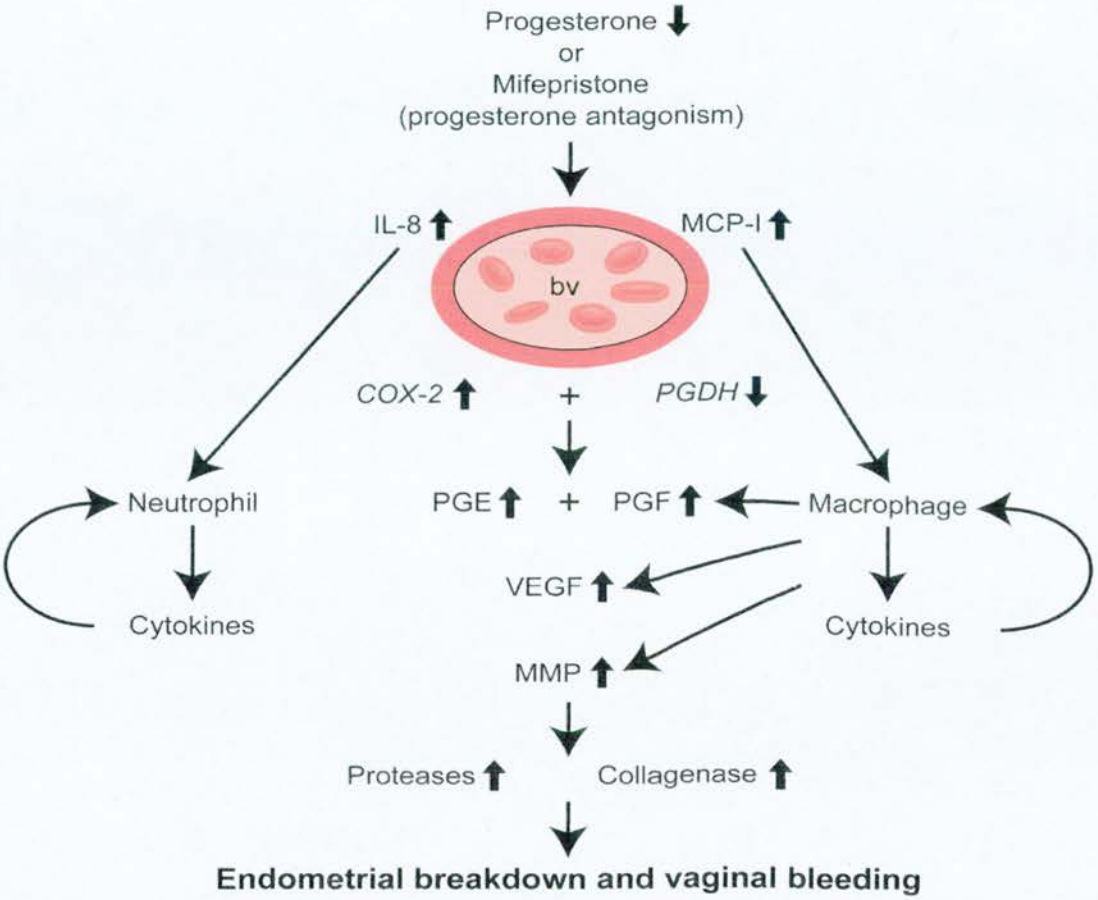


Figure 1.6 *Mechanism of menstruation.* Adapted from Baird (2000) with permission.

1.6.2.1 Prostaglandins and menstruation

Prostaglandins (PGs) are a family of fatty acids synthesised from arachidonic acid (AA) which are able to elicit a wide variety of biological actions. The amount of free AA available for cells is limited; therefore, liberation of AA from precursors (present as membrane bound phospholipids) by the action of phospholipase A_2 (PLA_2) is one of the first and rate limiting steps in PG synthesis (Lapetina 1982; reviewed in Poyser 1992). PLA_2 appears to be present in the endometrium in two different iso-forms; the calcium dependent and inducible $PLA_{2(i)}$ which is localised in the endometrial glands, and the calcium independent $PLA_{2(ii)}$ that is predominantly confined to the stroma (Bonney 1987

dependent and inducible $PLA_{2(i)}$ which is localised in the endometrial glands, and the calcium independent $PLA_{2(ii)}$ that is predominantly confined to the stroma (Bonney 1987 a & b). Free AA is then either converted to cyclic endoperoxides by the actions of cyclooxygenase or to 5-hydroperoxy acids by the action of 5-lipoxygenase (Figure 6.1). The end products of the cyclooxygenase pathway include prostanoids (PGE_2, $PGF_{2\alpha}$, PGI_2) and thromboxane A_2 (Bergstrom 1968; Poyser 1992; Baird 1996). $PGF_{2\alpha}$ is a strong vasoconstrictor and a stimulant of uterine contraction. Cyclo-oxygenase is a cytoplasmic enzyme (bound to the endoplasmic reticulum) and exists as two isoforms produced by two different genes (COX-1 and COX-2) (Fletcher 1992). COX-I is constitutively expressed in both glandular and stromal cells of the endometrium, while COX-2 expression is modulated by a variety of stimuli including growth factors and hormones and is usually limited to luminal, and glandular epithelial cells. In the endometrium, PGs are not stored, but are immediately synthesised and released, then rapidly metabolised into biologically inactive metabolites. The major site of prostaglandin synthesis during the normal menstrual cycle appears to be the endometrial glandular cell compartment (Lumsden 1984). The NAD+ dependent enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH) belongs to the family of short chain dehydrogenase and is responsible for the metabolism of PGs (Ensor 1990). The endometrial release of PGs varies at different stages of the menstrual cycle, which suggests an ovarian hormonal influence.

The evidence (both in vitro and in vivo) for progesterone control of endometrial PG activity are as follows; secretory endometrium has a higher level of PGs (both PGE_2 and $PGF_{2\alpha}$) (Downie *et al.* 1974; Sing *et al.* 1975; Maathuis & Kelly 1978) and has a greater capacity to synthesise PGs than the proliferative endometrium (Abel & Baird 1980; Smith *et al.* 1981); in-vitro, progesterone suppressed both the oestradiol-stimulated and basal PG production by endometrial explants and cells in culture (Abel & Baird 1980; Schatz *et al.* 1986; Kelly & Smith 1987); large amounts of PGs are released with the withdrawal of progesterone e.g. menstruation and abortion (Lumsden *et al.* 1983;

Norman *et al.* 1991 a & b; Cheng *et al.* 1993 b; Poyser 1995); during pregnancy, when progesterone levels are high, basal endometrial PG production is reduced (Maathuis & Kelly 1978); antagonising progesterone in vitro shows a dose-dependent induction of PGF2α release from endometrial stromal cells (Kelly *et al.* 1986); the observed increase in uterine contractility after treatment with mifepristone is possibly due to increase PGs (Norman *et al.* 1991b; Gemzell-Danielsson *et al.* 1994). Therefore, under normal conditions at least, progesterone appears to enhance the PG biosynthetic capacity of the secretory endometrium, while suppressing their release. However, mifepristone still increases the uterine activity even when prostaglandin production is suppressed by indomethacin (Norman *et al.* 1991a).

The concentration of PG in any tissue is related to its rate of synthesis and metabolism. Thus, attempts have been made through evaluating the enzymes involved to demonstrate the mechanism by which progesterone controls endometrial PGs activity. An increase in PGDH expression (Casey *et al.* 1980; Kelly *et al.* 1994), and a decrease in the expression of COX-2 (Jones *et al.* 1997) is seen with the rising levels of progesterone in the secretory endometrium; progesterone has an inhibitory effect on the endometrial PLA_2 activity (Bonney *et al.* 1987 a & b); the withdrawal of progesterone from an endometrium that has been primed with progesterone and oestradiol results in an increase in COX-2 expression (Critchley *et al.* 1999), while continuing exposure to progesterone is associated with low levels of COX-2 expression; following treatment with antiprogesterone, glandular PGDH expression is markedly reduced (Cameron *et al.* 1996); glandular PGDH immunostaining in the endometria of women receiving mifepristone in early pregnancy also shows a reduction (Cheng *et al.* 1993a) while it is increased in the endometria of women using a LNG releasing intrauterine system (Critchley *et al.* 1998a); and finally, transfection studies have shown progesterone induced PGDH expression via interaction with a specific promotor (Greenland *et al.* 2000). Thus, progesterone is responsible for stimulating PGDH and suppressing COX-2 and PLA_2.

Consequently, although progesterone enhances the PG biosynthetic capacity of the endometrium, withdrawal of progesterone is necessary for its full potential to be evident. Moreover, progesterone appears to exert these effects through inhibiting enzymes involved in the synthesis of PGs (PLA_2 and COX-2) and by stimulating PGDH, which metabolises PGs.

1.6.3 Mifepristone and the uterus

Mifepristone blocks the specialised uterine actions of progesterone firstly enabling the reception of the blastocyst by the endometrium and the continuous nourishment of the developing fetus and secondly containing the growing fetus until the eventual expulsion.

1.6.3.1 Effects of mifepristone on the endometrium

The endometrial effects are dependent on the dose of mifepristone used and on the time of administration in the menstrual cycle. In several studies mifepristone was tested in doses that varied between 0.5 mg and 800 mg, given either as single dose, multiple doses intermittently or continuously for several months.

Although an anti-proliferative effect has been reported in the absence of progesterone (Slayden & Brenner 1994; Brenner & Slayden 1994; Elger *et al.* 2000), mifepristone in general does not directly affect the endometrium. The persistent proliferative activity observed following follicular phase administration is a result of the delay in ovulation (Swahn *et al.* 1988). However, the endometrial effects of luteal phase (progesterone dominant) administration of mifepristone are numerous.

The endometrial effects of early luteal phase administration of antiprogesterones have been extensively investigated (Swahn *et al.* 1990; Gemzell-Danielsson *et al.* 1996; Cameron *et al.* 1996). Early luteal phase administration of a single or multiple doses of mifepristone disrupts endometrial maturation without affecting vaginal bleeding patterns. Treatment with early luteal phase mifepristone chronologically retards the

development of the secretory endometrium with glandular contraction, reduced luminal secretions and a decreased number of vacuolated cells (Swahn *et al.* 1990; Gemzelle-Danielsson *et al.* 1994a). In the early luteal phase mifepristone inhibits progesterone induced down-regulation of PR and ER (Berthois *et al.* 1991; Gemzelle-Danielsson *et al.* 1994a; Cameron *et al.* 1997) while antagonising the progesterone action on endometrial markers such as PGDH, which are known to be progesterone dependent (Casey *et al.* 1980; Greenland *et al.* 2000). Moreover, PGDH has been postulated as a useful marker of the "closure" of the implantation window, and the effect of mid-luteal administration on such markers may add to our current understanding of potential contraceptive actions of mifepristone. Antiprogesterone also increase AR expression in both glandular and stromal compartments (Cameron *et al.* 2000; Slayden *et al.* 2001). Furthermore, when 200 mg mifepristone is administered immediately following ovulation, uterine PGF2α release (Gemzell-Danielsson *et al.* 1994b), the luminal expression of COX-2 (Marions *et al.* 1999), and the glandular expression of leukaemia inhibitory factor (LIF: a cytokine involved in implantation process) are significantly reduced, while the immunoreactivity of Ki67 (a nuclear marker of cellular proliferation) is increased (Cameron *et al.* 1997). The reduction in the endometrial metabolism of oestradiol (Maentausta *et al.* 1993), and the reduction in the markers of endometrial maturation (Gemzelle-Danielsson *et al.* 1994a) suggest that early luteal phase treatment with mifepristone inhibits the normal secretory transformation of the endometrium.

In the mid-luteal phase, mifepristone at a single dose of more than 50 mg induces menstrual bleeding within 72 hours (Greene *et al.* 1992; Schaison *et al.* 1985; Shoupe *et al.* 1987). Luteolysis was incomplete in two thirds of the subjects, and they experienced a further episode of vaginal bleeding at the expected time of menses. In the remainder there was complete luteolysis with only one episode of bleeding. Thus the vaginal bleeding observed after mifepristone without a persistent decrease in the circulating progesterone values, appears to be due to a direct effect on the endometrium. Observations similar to those seen before menstruation such as shrinkage of glandular lumen, leucocyte infiltration and vascular necrosis have been reported (Li *et al.* 1988;

Swahn *et al.* 1988). Johannisson *et al.* in 1988 suggested that the menstrual bleeding induced by mifepristone in mid-luteal phase is a direct endometrial vascular effect. Following treatment with 50 mg of mifepristone in the mid luteal phase (days 20 – 23), they described a significant reduction in the capillary luminal area and diameter associated with degenerative changes in the endothelial cells, which preceded the menstrual shedding. They interpreted the decreased capillary luminal area as vasoconstriction. These changes did not always accompany regressive changes in the adjacent stroma. This effect of mifepristone on the endometrium (at a time when the endometrial progesterone receptor level is relatively low) is poorly understood, and has hardly been investigated. Mifepristone may induce bleeding by increasing local concentration of prostaglandins, which are potent vasoconstrictors. Indeed, the evidence is available for similar action of mifepristone on local PG in the early pregnancy where it has been known to reduce the PG metabolising enzyme PGDH (Cheng *et al.* 1993a). Moreover, in the early-luteal phase progesterone values are relatively low, and as a consequence the PR and ER expression is maximal, while the converse is true for the mid-lueal phase of the cycle. Therefore, in the early luteal phase mifepristone may prevent the effects that are yet to be exerted by progesterone, whereas in the mid-luteal phase it may antagonise the actions of progesterone, which at that time appears to be the suppression of release of PGs.

When given as a daily pill, the threshold dose of mifepristone required to disturb the endometrial development appears to be 1 mg/day (Batista *et al.* 1992b; Croxatto *et al.* 1993). 5 mg weekly dose will also be sufficient to inhibit the normal secretory development of the endometrium (Gemzelle-Danielsson *et al.* 1996).

In the absence of progesterone, mifepristone induced agonist-like secretory changes in the oestrogen primed postmenopausal endometrium suggests it to be a partial rather than a pure antagonist (Gravanis *et al.* 1985).

1.7 Contraceptive potential of mifepristone

Progesterone is essential for the establishment and the maintenance of pregnancy, thus the anti-progesterone mifepristone has multiple potential anti-fertility actions. Mifepristone is potentially contraceptive by virtue of its effects either on the hypothalamic-pituitary-ovarian axis, and/or, by direct effects on the uterus. The hypothalamic-pituitary effects are particularly apparent in the follicular phase of the cycle, where mifepristone in large doses inhibits gonadotrophin secretion, consequently arrests follicular-growth and prevents or delays ovulation (Shoupe *et al.* 1987; Permezel *et al.* 1989; Stuenkel *et al.* 1990). The endometrial effects are largely confined to the luteal phase. Mifepristone is able to retard the normal endometrial development or to induce endometrial shedding depending on the time of administration in the luteal phase. Moreover, the recently reported actions of mifepristone on the embryo, sperm and tubal function, may also have additional contraceptive effects(Yang *et al.* 1994; Baldi *et al.* 2000; Spitz 2000).

The use of mifepristone in the following contraceptive options has been explored.

1.7.1 Occasional menstrual regulation

Some have suggested using mifepristone early in the pregnancy when mestruation is delayed (up to 6 weeks of gestation) as a method of contraception. Women, even after having sexual intercourse during the fertile period of the cycle do not fall pregnant each month. Therefore, a menstrual regulatory agent that has to be taken occasionally (only if the conception had occurred and menstruation is delayed) can be attractive to some. However, this approach of using mifepristone alone to interrupt a pregnancy in the first 6 weeks was neither very effective (success rates about 85%) nor acceptable to many women (Couzinet *et al.* 1990; Spitz *et al.* 1993a; Glasier *et al.* 1998). Some reports have suggested that if given in combination with a prostaglandin to women with menstrual delay of up to 11 days, mifepristone may have a role as an occasional 'menstrual regulator' (WHO 1995), while others have shown that this regimen is ineffective when

given at the expected time of menses (Dubois *et al.* 1988; Swahn *et al.* 1999). Although this method has an important practical implication in those countries that allow menstrual regulation even when abortion is prohibited, its legality or acceptability in general is likely to be limited.

1.7.2 "Once-a-month" luteal phase administration

Mifepristone may prevent implantation in luteal phase either by preventing the development of the normal secretory endometrium, or by inducing endometrial shedding (mid luteal phase). When administered immediately following ovulation, although able to alter the endometrial development causing a delay in the emergence of the implantation window, a single-dose of mifepristone 200 mg did not affect the menstrual cycle length (Swahn *et al.* 1990). Preliminary results of clinical trials have confirmed the contraceptive efficacy of this approach. In the first study, after taking 200 mg of mifepristone on day LH+2 only one pregnancy occurred in 124 cycles, in which women were exposed to the risk of pregnancy (Gemzelle-Danielsson *et al.* 1993). Although many women find this approach to be an attractive option, the feasibility of administering mifepristone in this manner is deterred by the requirement of precise detection of the LH surge. An easy, highly effective, and cheap method to define the LH surge (probably in a urine sample) would be necessary for this approach to be suitable for wide-spread use.

1.7.3 "End of the month" regular late luteal phase administration

Although a simpler, and more practical approach to the above, since it will induce menstrual bleeding at the expected time of the month (Croxatto *et al.* 1987), the existing data on the contraceptive efficacy of mifepristone as a regular 'end of the month pill' in the late luteal phase are disappointing. Reported failure rates of approximately 5% per treatment cycle and 17% - 19% per pregnancy cycle (Ulmann *et al.* 1987; Dubois *et al.* 1988; Couzinet *et al.* 1990; Reviewed in Swahn *et al.* 1996b), are comparable to the failure rates of mifepristone when used on its own to terminate early pregnancy.

Addition of 400 μg misoprostol to 200 mg mifepristone used regularly at the expected time of menses did not improve the success rates (Swahn *et al.* 1999).

1.7.4 Emergency contraception

In theory mifepristone approximates the 'ideal' emergency contraceptive. If unprotected intercourse occurs before ovulation it is able to prevent pregnancy by delaying ovulation. Conversely, if unprotected inctercourse occurs after ovulation, mifepristone may prevent implantation via its endometrial action. Two randomised trials comparing 600 mg of mifepristone with the standard Yuzpe regimen of combine oestradiol and Levonorgestrel have confirmed its efficacy as an emergency contraceptive, when given within 72 hours of unprotected intercourse (Webb *et al.* 1992; Glasier *et al.* 1992). They also reported a significantly low incidence of side-effects with mifepristone. However, the main draw-back with this dose is the delay in the onset of subsequent menses, presumably when it was administered in the follicular phase. Reassuringly, a recent large multi-centre study comparing 10, 50, or 100 mg doses of mifepristone reported that the delay in the onset of the next menses and the side effects are dose-related, and the reduction of the dose to 10 mg did not compromise the efficacy (WHO 1999).

1.7.5 "Once-a-week" use

Weekly doses of either 2.5 mg or 5 mg mifepristone can delay endometrial maturation and the appearance of progesterone dependent markers of the endometrium, without affecting ovulation, hormonal profile or the menstrual cycle (Gemzell-Danielsson *et al.* 1996). However, in unprotected women the regimen of 5 mg every week failed to demonstrate efficacy in preventing pregnancy (Marions *et al.* 1998). When the weekly dose is increased to 10, 25 or 50 mg a considerable disruption of the menstrual pattern was observed with inconsistent inhibitory effect on ovulation (Spitz *et al.* 1993b & 2000; Chen *et al.* 1997). In view of this data, intermittent administration of mifepristone has been abandoned as a potential contraceptive method.

1.7.6 Daily low dose and cyclical administration

Continuous daily doses of above 2 mg of mifepristone reproducibly suppresses ovulation (Ledger *et al.* 1992; Corxatto *et al.* 1993) and, clinical trials have confirmed its contraceptive effect (Brown *et al.* 2002). A 5 mg dose consistently inhibits ovulation, while ovulation was permitted in 10% of the cycles with 2 mg dose. However, probably due to the additional anti-fertility effect on the endometrium, both doses are effective as contraceptives (Ledger 1992; Corxatto *et al.*1993). When women either took 2 or 5 mg mifepristone daily as their only method of contraception, there were no pregnancies in the 200 months of treatment (Brown *et al.* 2002).

Cyclical daily regimens of mifepristone in doses of 5 or 10 mg for the first 15 days of the cycle followed by a progestin for the subsequent 14 days had been tested with the view to produce a more 'natural' regular bleeding pattern. Although this regimen resulted in a regular bleeding pattern as expected in a significant number of patients, it did not suppress ovulation (Croxatto *et al.* 1996 & 1998). Furthermore, the contraceptive efficacy of this approach remains to be investigated.

1.7.7 Male contraception

Mifepristone opposes the effects of progesterone on calcium influx, sperm motility and the acrosome reaction by inducing a rapid, transient, and dose dependent decrease of intracellular free calcium and a drop in the fraction of hyperactivated sperm (Yang *et al.* 1994). This approach however, has not been pursued because of difficulty in reproducing these results.

1.7.8 Other clinical uses of mifepristone

Mifepristone has been widely used for pregnancy termination in the first and second trimester and it is licensed for this use in many countries in the world. The other possible uses of mifepristone include induction of labour, cervical ripening prior to suction termination of pregnancy or surgical intervention of the uterine cavity such as

hysteroscopy, for the treatment of endometriosis, fibroids, and progesterone receptor positive breast cancer. Potential non-gynaecological uses of mifepristone include reduction of intra-ocular pressure in glaucoma and steroid induced myopathy, treatment for PR positive brain tumours such as meningioma, and Cushing's disease due to ectopic ACTH secretion or adrenal carcinoma. These effects reported are believed to be secondary to its anti-glucocorticoid properties (Sakar 2002).

1.8 Emergency Contraception

Emergency contraception is defined as the use of a medication or a device to prevent pregnancy once sexual intercourse had occurred (Glasier & Baird 1997) yet many perceive EC as abortifacients. This is because the action is taken after, rather than before intercourse. However, the definition of an abortion is interference with a pregnancy after implantation and, the time from ovulation to implantation takes an estimated seven days. Since the ovum, once ovulated, is capable of being fertilised for only up to 24 hours, use of an EC within 72 hours of sexual intercourse (that is responsible for fertilisation) cannot induce an abortion. Nevertheless, this confusion and misinterpretation among the providers and the users of EC may be the main impediment to the widespread use of emergency contraceptives, which could prevent millions of unwanted pregnancies in the world (Trussell *et al.* 1992). A detailed understanding of the precise mechanism by which an emergency contraceptive prevents a pregnancy can be advantageous in clarifying these misconceptions.

Unfortunately, the exact mechanism of action of most widely used emergency contraceptives is still poorly understood.

1.8.1 Methods available

Not using a contraceptive at all or failures in a method used (e.g.: burst condom) are common reasons for a woman to seek emergency contraceptives. Today, the available methods of post-coital contraception in the UK include Yuzpe regimen of a combination of oestrogen (ethinyl oestradiol) and progestin (Levonorgestrel), progestin alone

(Levonorgestrel) and the emergency insertion of intrauterine device (IUD). Although the methods involving steroid hormones eclipse the highly effective yet inconvenient IUD in post coital contraception, they are associated with high incidence of side effects. The data available on the safety of these regimens when used as EC are limited, yet at least theoretically they appear to be extremely safe. All the above regimens can be used as long-term contraception and there is an abundance of data indicating their safety when used in these ways (reviewed by Turner & Ellerston 2002). Furthermore, WHO and the US Food and Drug Administration, have declared that EC has an excellent safety profile in nearly all women. In their review of EC safety, Turner *et al.* went on to suggest that whether carried to term or terminated, an unwanted pregnancy is more hazardous to the well-being of the woman than the risks posed by taking EC.

1.8.2 Efficacy of EC

Generally efficacy of EC is defined as how well the method prevents pregnancies in ideal conditions. There are no placebo-controlled trials quantifying the efficacy of emergency contraceptives. Conventionally, the effectiveness of emergency contraceptive methods have been calculated by comparing the observed number of pregnancies with the number expected in the absence of treatment (Yuzpe & Lancee 1977; Ho *et al.* 1993; Glasier *et al.* 1997; WHO 1998). In most studies to date, the anticipated number of pregnancies is estimated by using external data based on two studies of couples planning pregnancy (Barrett & Marshall 1969; Wilcox *et al.*1988). The two studies calculated the probability of pregnancy resulting from unprotected intercourse on a particular day of the cycle, relative to the predicted day of ovulation. This approach poses some obvious problems. Only women with regular cycles can be used to estimate the efficacy; the validity of the results is directly dependent on the accuracy of the reported menstrual data; the estimates are sensitive to errors in identifying the day of ovulation (Dunson *et al.* 1999); other factors can affect the probability of pregnancy (e.g. was there an actual spillage of semen, fertility of the couple) that are unrelated to the timing of intercourse (Schwartz 1980) and conception is used synonymously with implantation, not

fertilisation (Trussell *et al.* 1998). It has been suggested that the true effectiveness of EC may be higher than the calculated figure for the following reasons; the estimated number of pregnancies tend to be smaller since the estimates are based on studies which are deficient; the number of pregnancies observed tend to be higher due to women either being pregnant at the time EC was administered or they become pregnant by a subsequent act of intercourse (Trussell *et al.* 1998). Conversely, the argument remains that the effectiveness has been overestimated because the risk of pregnancy without EC is less than that calculated from Dixon / Wilcox tables. The women in Dixon / Wilcox estimates were trying to get pregnant, without any history of infertility and had regular menstrual cycles. Many women who present for EC are not at risk because they are nowhere near ovulation, and they may not have had vaginal intercourse with sperm in the vagina. Thus the effectiveness of EC may be overestimated.
Table 1.1 illustrates the reported efficacy of the different methods.

1.8.3 Mechanism of action of emergency contraceptives

An emergency contraceptive can be effective by inhibiting or disrupting ovulation / fertilisation, interfering with the transport of the blastocyst, or inhibiting its implantation in the endometrium. Sex steroids have several effects on the ovulatory cycle and on the endometrium depending on the time of administration, hence have been used as emergency contraceptives.

Today, a quarter of a century since its initial discovery, the mechanism by which Yuzpe regimen (Yuzpe & Lancee 1977) acts as an EC remains unclear. Although different theories are on offer, based on reported effects after administering the Yuzpe regimen such as, interference with ovulation (Ling *et al.* 1983a; Rowlands *et al.* 1986; Swahn *et al.* 1996a; Croxatto *et al.* 2002), or disrupting endometrial maturation (Ling *et al.* 1983b), they appear to be insufficient to fully explain the observed clinical efficacy of the regimen (Trussell & Raymond 1999). The minor changes observed in the endometrium

Table 1.1 The effectiveness of available methods of emergency contraception

Regimen	Time after intercourse	Reported efficacy	Source of data
Yuzpe	Within 72 hours	72.5-74.1% of expected no of pregnancies are prevented	Meta-analysis of 7 and 10 studies (Trussell 1998; Trussell 1999b)
LNG 0.75 mg taken twice 12 hours apart	Within 72 hours	Similar to Yuzpe	2 randomised trials involving a total of 1386 women (Ho 1993; WHO 1998)
Mifepristone Single dose of 10 – 600 mg	Up to 120 hours	85% to 100%	3 randomised trials involving a total of 2281 women (Glasier 1992; Webb 1992; WHO 1999)
Danazol 400 - 800mg x2 or 400mg x 3 taken 12 hours apart	Within 72 hours	failure rates of <1% to 100%	2 randomised trials, Webb 1992 (n= 193) shows no effect. Zuliani 1990 (n >1700) shows <1% failure rate
Copper intra uterine device	Up to 5 days after the earliest estimated day of ovulation	Failure rate <1%	Meta – analysis of 20 published studies involving more than 8000 women (Trussell 1995)
High dose Oestrogen (e.g. 5mg per day ethinyl-oestradiol for 5 days)	Within 72 hours	Similar to Yuzpe	Randomised trial involving 250 women (Dixon 1980)

during the implantation period following Yuzpe {changes in endometrial ER and PR (Kubba *et al.* 1986), MUC-1 expression (Raymond *et al.* 2000), minor morphological changes (Raymond 2000)} are inconsistent (e.g Swahn *et al.* 1996b; Marion *et al.* 2002) and may even be incapable of preventing implantation.

1.8.4 LNG as an emergency contraceptive

Two consecutive doses of Levonorgestrel (LNG) 0.75 mg administered 12 hours apart within 72 hours of the unprotected coitus, has been shown to be an effective method of post coital contraception (PCC). This regimen acquired a licence as an emergency contraceptive in the UK during the latter part of the year 2000. It is also being used for this purpose in Eastern Europe, the Far East and many developing countries. There have been only 2 trials (Ho *et al.* 1993; WHO 1998) published to date comparing the efficacy of this regimen with the standard method of emergency contraception, the Yuzpe regimen. The two studies showed that LNG performed effectively as an emergency contraceptive and had fewer side effects than the Yuzpe regimen. Neither of the two studies was designed to identify the possible mechanism(s) of action by which LNG exerts its post-coital contraceptive effect. Hence, the mechanism of action of LNG used in this manner remains unknown. Furthermore, in some countries, introduction of EC requires evidence on mechanism of action that suggest further exploration into the effect of LNG would be advantageous.

1.8.5 Possible EC mechanisms of action of LNG

1.8.5.1 Effect of LNG on the LH surge, ovulation & luteal function

In physiological doses progesterone initially promotes the oestrogen elicited mid-cycle LH surge in oestrogen treated ovariectamised women (Odelle & Swerloff 1968), and in women with intact ovaries (Permezel *et al.* 1988). Long-term administration however, enhances the negative feedback effects of E2 (Dierschke *et al.* 1973; Clifton *et al.* 1975; March *et al.* 1981). In combination with oestrogen, continuous administration of progestin (in the form of COCP) abolishes the pre-ovulatory LH surge and suppresses

the LH levels down to those observed during the luteal phase (Swerdloff *et al.* 1968; Klopper *et al.* 1973). The Yuzpe regimen also has been shown to suppress or delay the LH surge and ovulation (Croxatto *et al.* 2002). On their own, synthetic progestins (Norgestrel) at supra-physiological doses have been shown to abolish the mid cycle LH surge leading to anovulation and delay of the onset of the subsequent menses (Weiner *et al.* 1975). The lowest plasma level reported to inhibit ovulation appears to be 1 nmol/L (Nilsson *et al.* 1980). Repeated supra-physiological doses of (0.75 mg) LNG in the peri-ovulatory phase of the cycle delayed the LH surge and disrupted ovulation and normal luteal function in some women (Landgren *et al.* 1989). Others have also reported a decrease in the mid-luteal LH surge after treatment with LNG in the follicular phase, with the exception of some women this was associated with a normal luteal phase pregnanediol excretion (Kessaru *et al.* 1974; Sopna *et al.* 1975; Marions *et al.* 2002). Progestins suppressed FSH induced E2 production by rat granulosa cells in culture (Schreiber 1980; Fortune 1983), decrease synthesis of oestrogen pre-cursor (androstendione) and inhibit follicular development in monkeys, despite FSH enhancement (Goodman *et al.* 1982; Weibe *et al.* 1984). These observations suggest that the pre-ovulatory administration of LNG may interfere with the E2 signal that precedes the LH surge.

During the luteal phase of the cycle, progestins depress basal LH secretion (Weiner *et al.* 1975). In the human, basal level of Lutenizing Hormone (LH) is essential for the normal secretory function of the corpus luteum (Vande Weile *et al.* 1970). In 1971 Johansson *et al.*, suggested that synthetic progestins may have a possible post ovulatory contraceptive effect by interfering with normal luteal function and decreasing plasma progesterone. Many have reported a shorter luteal phase after progestin administration, which accompanied a lower than usual luteal progesterone production (Johansson 1971; Kessaru *et al.* 1975; Durand *et al.* 2001).

Table 1.2 illustrate the effects of synthetic progestins on the menstrual cycle reported in the literature.

Table 1.2 Effects of synthetic progestogens on the menstrual cycle

Reference (n = subjects)	**Progestin / Dose**	**The cycle day (cd) of administration**	**Results**
Weiner *et al.* 1980 (n = 4)	d-norgestrel 40 mg implant	Day 5 onwards 90 + days	Ovulation suppressed LH peak suppressed
Landgren *et al.* 1990 (n = 20)	Norethisterone 300 mcg (n = 10) LNG 30 mcg (n = 10)	Day 7 - 10	**NET;**In 4 both LH peak and pregnanediol supressed. Follicular phase was prolonged in one; area under the curve (AUC) increased for E2, decreased for Pregnanediol (P_4); Endometrial sub-nuclear vacuolation and decreased gland mitosis **LNG**; No LH peak in 3 and pregnanediol supressed in 4. One woman had a prolonged follicular phase. Decreased AUC of P_4 in all No endometrial effects
Landgren *et al.* 1989 (n = 72)	0.75 mg LNG 1. n = 17 2. n = 17 3. n = 18 4. n = 19	1. 2, 4, 6 and 8 2. 9, 11, 13, and 15 3. 11, 12, 16 and 19 4. 16, 18, 20 and 22	1. 17/17 normal cycle 2. 3/17 anovulatory, 7/17 incomp luteal activity, 7/17 normal 3. 5/18 anovulatory, 6/18 incomp luteal activity, 7/18 normal post-treatment cycle 1/18 anovulatory, 1/18 incomplete luteal activity 4. 19/19 normal cycle

Johansson *et al.* 1971 (n = 7)	Norethisterone Norgestrel MPA Chlormadinone	All post-ovulation LH+3 onwards	Shortened luteal phase Reduced total P_4
Kesseru *et al.* 1974 (n = 11)	d-norgestrel 400 mcg	**Group A** 1. 10 (n = 4) 2. 6 & 10 (n = 4) 3. 6,10,14 (n = 1) 4. 6,10,14, 18 (n=2) **Group B (n = 9)** PCC tablets 5 – 12 per subject	**Group A** 1. 1/4 no LH peak } All had 2. 3/4 no LH peak } normal luteal P_4 3. & 4. Both decreased LH peak } levels **Group B** 7 showed no LH peak, significantly low P_4 levels if >5 tablets were taken. Intra-cervical sperm penetration effect seen 9 – 10 hours (h) after ingestion. Cervical mucus changes starting at 3-4h and max change at 9h post-ingestion.
Craft *et al.* 1975 (n = 8)	Norgestrel 750 mcg	Follicular phase up to 9 doses	7 had delayed menses, and showed no LH peak or delayed LH peak. All 9 women also reported irregular vaginal spotting. One had short luteal phase.

Spona *et al.* 1975 (n = 6)	d-norgestrel 400 mcg	1. 12 2. 10 & 12 3. 10, 12 & 14 4. post ovulatory	1. No LH peak but normal rise in P_4 2. Abnormal LH rise and short luteal phase 3. No LH peak, delayed rise in P_4 (may suggest delayed ovulation) 4. No effect
Durand *et al.* 2001 (n = 16)	LNG (EC regimen)	Group A(n =15, d 10) Group B & C (n = 11 during LH peak) Group D (n = 8, late follicular phase)	A = 12 anovulatory, remaining 3 short luteal phase and low luteal P_4. B and C = no effect D = low luteal P_4, no effect on LH peak
Marions *et al.* 2002 (n = 12)	LNG (EC regimen)	Administered on LH-2	Low LH peak, normal luteal P_4 One short luteal phase.

1.8.5.2 Effect of LNG on endometrium

The main effects of short-term administration of LNG on the endometrium seem to be secondary to the delaying of ovulation. Landgren and colleagues (Landgren *et al.* 1989) showed that follicular phase administration of multiple doses of 0.75 mg LNG suppresses the secretory activity of the endometrium. However, the same dose of LNG did not affect the endometrium when given in the luteal phase. Glandular atrophy, stromal decidualisation and epithelial cell inactivation have been observed after chronic local exposure of the endometrium to LNG (Pakarinen *et al.*1995). Locally administered LNG also induces expression of the potent contraceptive glycoprotein Glycodelin A during the implantation period (Mandelin *et al.* 1997). At high doses (20 – 40 ng/ml) progesterone inhibits the growth of human capillary endothelial cells in culture (Peek *et al.* 1995). Similarly, although LNG stimulates angiogenesis in the dose range 100 pmol/l to 10 nmol/l, this effect was reversed at higher doses (Hague *et al.* 2002). Recently, Marions *et al.* reported that the emergency contraceptive dose of LNG administered in the peri-ovulatory period did not change any of the endometrial parameters including integrin $\alpha 4$ and $\beta 3$ (these are surface receptors for extracellular cell adhesion proteins to cytoskeletal components and are involved in blastocyst implantation), COX 1, COX 2, progesterone receptors, pinopodes and *Dolicos biflorus agglutinin* lectin binding (illustrates the secretory activity of the endometrial cells) (Marions *et al.* 2002). Therefore, the question remains as to whether any alterations occur in the endometrium after taking the emergency contraceptive regimen of LNG at other times in the cycle, and whether those changes are sufficient to prevent implantation and contribute to the observed contraceptive efficacy of LNG used in this manner.

1.8.5.3 Effect of LNG on cervical mucus

The endocervical mucosa of the human uterine cervix consists of pockets of columnar epithelium (comprises different types of secretory cells) forming the so called 'crypts' that vary in structure with age, disease and stage of the menstrual cycle. The secretions from these cells (principle component of the cervical mucus) are regulated by the

circulating ovarian hormones, thus show a cyclical variation. Oestrogens stimulate secretion of acellular, elastic, watery mucus, and progesterone blocks the oestrogen-induced secretory activity of the cervical mucosa to produce a cellular, viscous, scanty mucus that is hostile to sperm. It has been proposed that cervical mucus plays several key functions including: aiding sperm penetration at or near ovulation while interference with entry at other times; protecting sperm from the hostile vaginal environment; supplying nutrients; selecting sperms (by working as a filter) on the basis of differential motility; storing sperm; and initiating sperm capacitation (WHO laboratory manual 1992). The effect of progestagens on cervical mucus and on the cervix is well documented and this is thought to be the main mechanism by which the progestagen only pill exerts its anti-fertility action (Odeblad *et al.* 1972; Daunter *et al.* 1976; Moghissi *et al.* 1973). However, to be of an emergency contraceptive significance, these effects require treatment to be initiated relatively early once intercourse has taken place. The transportation of sperm from the vagina to the fallopian tube seems to be extremely rapid (Kuntz *et al.* 1996), so even if LNG has an effect on cervical mucus that interferes with sperm penetration, that action is unlikely to prevent a pregnancy when taken some 12 - 72 hours after coitus.

1.8.5.4 Effects of LNG on the sperm

Although it is widely accepted that progesterone has an important role in sperm function, the precise mechanism by which it exerts this action is not known. In order to fertilise the ovum, the spermatazoa is required to penetrate through the cumulus mass and progesterone is a main secretory product of the cumulus matrix. Progesterone stimulates a rapid influx of calcium into the spermatozoon via a non-genomic action (Plant *et al.* 1995; Tesarik *et al.* 1996; Aitkin *et al.* 1996), induces an acrosomal reaction (Oehninger *et al.* 1994), and increases hyperactive motility of the sperm (Uhler *et al.* 1992). An impaired response of the spermatozoon to progesterone is a clinically well-recognised defect in the infertile male (Tesarik *et al.* 1992; Falsetti *et al.*1993). Given their total lack of transcriptional ability, it would appear that in the sperm the non-genomic

progesterone receptor would have to be responsible if LNG were to have an emergency contraceptive effect.

1.9 Patient Compliance

1.9.1 Definitions and history

Generally, compliance is defined as yielding; complaisance; or submission (Chambers dictionary). In a clinical context, it is described as following the instructions of the health care professional (Haynes 1979) and is synonymous with adherence; maintenance; fidelity and concordance (Sackett 1976). Most of these definitions denote obedience – "following doctors orders". Therefore, the word "compliance" can be associated with somewhat 'negative' connotations for the patient. If the patient perceives the very concept of compliance as one that is morally and psychologically flawed, non-compliant behaviour can be as deviant as compliance in itself (Mullen, BMJ editorial 1997). However, to the prescriber, these definitions explain the patient's behaviour and, also describe the relation between prescription and the clinical outcome (Eisen 1991).

Patient adjusting or discontinuing the therapeutic regimen without professional medical advice is not a novel concept. Rather, it dates back to the period before Hippocrates (Wright 1993). Therefore, human nature may be at blemish for individuals selecting to follow the many deviations in the prescribed therapeutic plan. Whatever the reason is, non-compliance has serious implications for drug regulation, research and medical care.

1.9.2 The magnitude of the problem of non-compliance

Medicines are rarely taken as prescribed (Marinker 1997). This may adversely affect the quality of medical care and may waste resources. Over 50% of the patients suffering from chronic disease do not take the prescribed medicines in doses sufficient to achieve the therapeutic effect (Sackett 1979). Reflecting on their own adherence behaviour, a group of health care professionals who formed a working party on medicine taking admitted that their behaviour rarely coincided with the prescribed medical advice

(Marinker 1997). In any medical discipline, non-compliance disrupts and mitigates the benefits of the preventative or curative regimen offered. Since the medical profession is reportedly poor in identifying non-compliers (Gilbert *et al.*1980; Bonner & Carr 2002), consequences of non-compliance can lead to unnecessary diagnostic tests or additional treatment procedures, thus generating further financial wastage. In 1995 Rosenburg and colleagues estimated $2.6 billion as the cost associated with unintended pregnancies that occurred due to poor compliance with the oral contraceptive pill.

Non-adherence to a protocol can have an undesirable influence on study power and interpretation of results. When undetected, poor compliance can invalidate results of efficacy studies (Rudd 1991; Urquhart 1991; Dominik *et al.* 1999).

Although many investigators have shown an active interest in the field of patient compliance since mid 1960's, the ambiguity and the divergence in their viewpoints have only confused our current understanding of the nature and the extent of the problem. Compliance has been described in terms of problems of omission (e.g.; failure to report an event, failure to take a medicine or to report an adverse effect) or commission (e.g.; inaccurately over reported events, overdose) (Green *et al.* 1991). It can be linked to the clinical outcome as either the cause (e.g.; a reason for a medicine that is effective for a certain population being labelled as ineffective) or the effect (e.g.; side effects of a treatment causing patients to be non-compliant with the regimen) (Hurley 1991). Patient and behavioural parameters (age, life style (Potter 1991; Rapoff & Barnard 1986; Meichenbaum 1987)), regimen parameters (dose interval, route of administration (Puller *et al.* 1988; Rapoff 1989; Cromer *et al.* 1989)) and response feedback parameters (side effects, positive drug effects) are recognised by some as the determinants of compliance (Haynes 1979; Hurley 1991).

Unfortunately, this renewed interest has not delivered the desired new concepts for improving patient compliance, yet it seems unfair to criticise the studies that explored

the issue of compliance for their poor methodology. There is a lack of sound techniques to quantify compliance. The existing knowledge of quantitative and qualitative features of non-compliance behaviour that is prerequisite for a 'good' study design has been limited. Thus future research should be directed towards rectifying these deficiencies. Otherwise, it is difficult to conjecture progress.

1.9.3 Measuring and monitoring compliance

There are direct and indirect methods of quantifying compliance yet limitations exist in all these methods.

1.9.3.1 Direct methods

Direct observation of the patient ingesting a medicine, biologic parameter affected by the medication and physiologic changes induced by the medication, therapeutic drug monitoring using blood or urine tests, unannounced spot checks on patients, and clinic attendance are used in various instances as methods to measure compliance directly (Spilker 1991).

Direct observations by the investigator is only suitable for inpatient studies and a few studies where the patients are required to attend the clinic to obtain infrequent doses of medicines. In our study patients took the 200 mg of mifepristone tablet under the direct supervision of the investigator. Even under these exceptional conditions, some patients can still be non-compliant (e.g.; pretend to put the medicine in the mouth but spitting it out later) (Spilker 1991). Furthermore, methods such as measuring biological markers and drug levels in biological fluids cannot disclose the chronic adherence behaviour and several other variables apart from compliance may affect pharmacokinetic values (Cramer 1990). ***Spot checks*** in particular are a potential invasion of patient's privacy.

1.9.3.2 Indirect methods

Patient self-report (SR), pill counts (PC), the frequency of obtaining repeated prescriptions and a variety of electronic devices (For example; devices that monitor self testing of daily urine or record removal of each tablet) have been used to ascertain compliance indirectly (Pullar 1988; Waterhouse *et al.* 1993; Simmons *et al.* 2000). Although SR remains the most widely used tool measuring compliance in clinical practice and in research, many have reported significant overestimation of compliant behaviour with SR (Potter *et al.* 1996; Waterhouse *et al.* 1993; Simmons *et al.* 2000). A group of OC users self reported missing only 34% of the pills, when the data from an ***electronic device*** with diary entries showed 75% of the pills being missed (Potter 1996). Similarly, when compared to a micro-electronic monitoring system, traditional measures (SR and pill counts) significantly over estimated the adherence behaviour in a group of women receiving oral tamoxifen for treatment of invasive breast cancer (Waterhouse *et al.* 1993).

Anyhow, finding a method that measures compliance efficiently and that can be applied universally is just the beginning. One has to then, find a reference point in compliance, to discern compliers from non-compliers.

1.9.4 Non-compliance in research studies

Poor patient compliance is one of the most commonly overlooked problems in clinical research (Dahlstrom & Eckernas 1991). If undetected, it can make the results of a clinical trial invalid. Furthermore, the results from a compliant patient population may not be appropriate to extrapolate to a non-compliant group and vice versa (Dominik *et al.* 1999). The significance of the occurrence of non-compliance with a particular aspect of a trial depends on its relationship to the outcome. Though it is irrefutably wrong to overlook the issue of non-compliance in our approach to patient care, due to the complex nature of behaviours that involves, many doctors and investigators choose to ignore it completely.

Randomised controlled trials may not necessarily recreate or mirror clinical practice in general. They are highly artificial scientific endeavours hence; their interpretation is dependent on the possibility of the scientific principle being applied in the clinical setting (Hurley 1991). However, common sense suggests that the prevalence of non-compliant behaviour is likely to rise in the general population compared with that of a typical research study.

For a volunteer, taking part in a research study usually involves extra effort. Volunteers in clinical trials are required to attend extra clinics, answer questions, collect samples such as daily urine, allow invasive tests such as blood tests, and keep accurate records of daily events in order to comply with the study protocol. People volunteer for research studies for various reasons. Some may seek positive health benefits from a 'new' treatment in a study, and this may become their motivation to volunteer. However, in most randomised trials with a control arm and in preliminary biomedical studies the participants are aware that they may not experience a favourable effect from the treatment. They may expect other benefits; such as health screening, individual attention from health care professionals, first-hand knowledge into new discoveries, and financial gains; or they may just be altruistic. Anyhow, if the volunteer perceives the sacrifices necessitated by being involved in a research study to be more burdensome than their perception of the personal or other benefits of the study, non-compliance will be inevitable.

Even though non-compliers are more likely to withdraw or drop out from a study, they should not be excluded from the analysis of phase III trial results. The dropout rate may indirectly provide extra information on the true effect of a treatment regimen that can be expected in general use. In phase I trials however, non-compliance can affect the findings thus non-compliers may be withdrawn.

1.9.5 Compliance with contraceptives

Naturally, an improved understanding of the causes of non-compliance can aid in finding means to achieve better compliance. In order to introduce a new contraceptive method successfully, effort must be made to minimise "adverse events" and particularly side effects, in order to maintain a good level of compliance. What factors will interest women in a new method of contraception and encourage them to continue using it? Conversely, what will confound its success and dissuade women from using it? Inferences can be drawn from the information on acceptability of existing contraceptive methods among different groups. We can assume that life-stage, age, level of education and personal experience might play an important role in women's knowledge of and attitudes towards contraception. Moreover, the reliability, accessibility, and minimal interference with sex life may be major motives for the choice of contraceptive.

The psychosocial literature on health behaviour concludes that the "health belief model" is the best explanation for non-compliant behaviour undertaken by a healthy person (Kasl & Cobb 1966). If this model is applied to the contraceptive compliant behaviour, following key elements have to be recognised.

1. The users perception of their own susceptibility (probability) of pregnancy.
2. Their attitude towards pregnancy and the perception of severity of the consequences of pregnancy (physical, financial, psychological and social).
3. Their personal definition of compliance and understanding of the importance of perfect compliance.
4. Their perception of the effectiveness of the contraceptive method if properly used.

The users of contraceptive regimens are usually young and healthy and, for the individual, the serious outcome of non-compliance (pregnancy) is rare. They have to adhere to a contraceptive regimen on a long-term basis. They may find the daily requirement for medication taking as in oral contraceptive pills or daily urine testing as in Personal Hormone Monitoring device to be complicated or inconvenient. Side effects of the method may not be acceptable and, they may not have faith in the effectiveness of

the method. Considering these odds, it is not difficult to infer why compliance problems are frequent.

Most of the literature on contraceptive compliance is concentrated on the combined oral contraceptives (COC). For example, Emans and colleagues showed that among adolescents COC non-compliant behaviour was more pronounced (only 48% compliant) in inner-city teenagers attending a hospital clinic than among their suburban upper middle class counterparts (over 84% complaint with the COC) visiting a private clinic. Although several other patient characteristics have been associated with COC non-compliance (Rosenburgh *et al.* 1995a & 1995b), the relevance of these findings in other contraceptive use is open to question.

1.10 Detecting the fertile period of the cycle and ovulation

A simple and reliable means of ovulation detection will be invaluable in clinical practice as well as in research. In a clinical setting this information can be used by couples to time sexual intercourse in order to avoid or achieve pregnancy. Since the effects of sex steroids depend on the time of administration in the menstrual cycle, such information can assist in contraceptive research. An example would be the evaluation of the endometrial effects of peri-ovulatory administration of LNG.

The length of each menstrual cycle changes to a great extent in the same woman and also between women. The intra-subject and inter-subject variation of the length of the menstrual cycle is wide. This makes reliable prediction of the day of ovulation difficult. Estimates using clinical data have suggested the number of fertile days in each cycle to be around 6 days, ending on the probable day of ovulation (Wilcox *et al.* 1995). For years attempts have been made to identify potential markers of the fertile period, which would predict and detect ovulation. These include measurement of systemic changes that accompany the hormonal changes (basal body temperature (BBT) change & cervical mucus changes), assays of reproductive hormones in serum and urine, assays of cellular

and secretory elements of endometrium, trans-vaginal sonographic imaging of ovarian follicular activity, and sonographic evaluation of the vascular and morphological changes in the uterus.

1.10.1 BBT change

BBT at rest shows a detectable shift of $0.2\text{-}0.4^0$ C following ovulation due to the increasing production of progesterone from the corpus luteum. Hand-held electronic devices that utilise this temperature change to identify the end of fertile period have been tested claiming up to 94% agreement with the day of maximum follicular diameter as determined by ultrasound (Ismail *et al.* 1989; Flynn *et al.* 1991). However this method is neither prospective in estimating the day of ovulation, nor does it identify the onset of the fertile period.

1.10.2 Cervical-vaginal mucus or saliva changes

Under the influence of circulating sex steroids, the physical characteristics of the cervical mucus changes, and these changes are employed to prospectively identify the fertile period (WHO 1980) and to retrospectively estimate the day of ovulation (WHO 1983). NaCl in the saliva or cevico-vaginal mucus increases cyclically under the influence of oestrogens. This is responsible for the observed 'ferning' or crystallization pattern of these secretions that coincides with the fertile period in the cycle. A variety of small hand-held devices have been developed for the purpose of self-observing ferning patterns in either saliva or cervical- vaginal mucus. Small microscopes to determine the ferning in the context of other markers of fertility have been suggested with only limited success (Fehring *et al.* 1998).

Further attempts have been made to detect the cyclical volumetric changes in cervico-vaginal fluid (CVF), which correlate with levels of steroid hormones. By measuring the CVF volumes using a vaginal aspirator (Rovmeter) and correlating it with serial ultrasound measurements of the dominant follicle, Flynn *et al.* showed a consistent CVF

volume peak occurring the day before ovulation. They went on to demonstrate an abrupt fall in CVF volume following ovulation, which marked the onset of the infertile phase of the cycle (Flynn *et al.* 1988). Although the preliminary work suggests this an attractive, easy to use and inexpensive method of identifying the fertile period, further development and larger clinical studies are needed to substantiate these claims.

1.10.3 Hormone assays

It has been known for years that detecting the mid-cycle LH surge is the best marker of impending ovulation (WHO 1983). However, daily laboratory testing of serum or urinary LH did not offer a realistic means to be used in the community to detect ovulation in large scale. The subsequent development of enzyme-linked immuno-absorbent assay provided the answer with commercially available LH detection kits to test urine every 6, 12 or 24 hours (Garcia *et al.* 1981; Kasper *et al.* 1984; Hirsch *et al.* 1986; Vermesh *et al.* 1987). These were simple to use at home, and were rapid and reliable in detecting the mid-cycle urinary LH surge. However, the LH peak in urine precedes ovulation by approximately 24 - 48 hours (WHO 1980; Collins *et al.* 1996), hence if only LH was used to predict impending ovulation, the possible number of days of advanced warning achieved would be limited to two.

Further studies have investigated the possibility of incorporating information from rising levels of oestradiol that accompany the development of the dominant follicle and precede the LH surge, to make the warning of onset of the fertile period to be of more realistic length. Several authors identified a positive correlation between the early morning urinary levels of oestrone-3-glucuronide (E3G), the principal metabolite of oestradiol and the serum levels of oestradiol (Branch *et al.* 1982; Catalan *et al.* 1989). A rise of >50% of urinary E3G concentration over the mean value of the previous 3 days will forewarn the onset of the fertile period in between 83% - 90% of the ovulatory cycles (Aldercreutz 1982; WHO 1983; Schiphorst *et al.* 1985). Others have suggested

that it may be possible to use the urinary levels of progesterone metabolites to mark the end of the fertile period (Blackwell *et al.* 1998).

In 1999 Bonner and colleagues reported the development of a hand-held device (Personal system of Contraception or Persona®) as an aid to avoid sexual intercourse during the fertile period, and in 2000 Behre *et al.* described a similar system (ClearPlan® Fertility monitor) to be used for the opposite effect, i.e. to maximize the chance of conception by timing intercourse to the fertile period (Bonner *et al.* 1999; Behre *et al.* 2000). Both systems utilise information from E3G and LH levels in early morning urine to predict the fertile period and to detect ovulation, although they employ a different monitors, software and urine test sticks. It is fundamental that the algorithms utilised to warn of the fertile period in the two systems are different, since the contraceptive device requires a narrower margin of error and therefore indicates warning of potential fertility for at least 7 days (median 11, range 7 - 17 days), while the opposite is true for the pro-conceptive device in order to aid conception (median warning 6 days, range 0 - 20). The reported detection rate of LH peak (within 2 days of monitor indicated day of peak fertility) is around 91.1%.

1.10.4 Endometrial secretory elements

Biochemical tests of changing concentrations of cellular and secretory constituents of the endometrium that occur in response to ovarian hormones have been used to detect ovulation. Seif *et al.* reported the use of a monoclonal antibody to a cycle-dependent sialo-glycoprotein (Seif *et al.* 1989) to identify the peri-ovulatory period. Clearly, more studies in this direction are needed.

1.10.5 Ultra-sonography

For over two decades ultrasound has been used to monitor follicular growth and to identify ovulation (Queenan 1980; Vargyas, 1982). The reported ultra-sonic indices of ovulation include disappearance or sudden decrease of follicle size, appearance of

ultrasonic echoes in the follicle, irregularity of follicular wall, and appearance of free fluid in the pouch of Douglas (Ecochard *et al.* 2000). Disappearance of the dominant follicular diameter appears to be the most sensitive of the indices with over 84% specificity and nearly 90% sensitivity (Vermesh *et al.* 1987; Behre *et al.* 2000; Ecochard *et al.* 2000). The urinary LH surge is also associated with ultrasonic changes in the uterus, including a continuous rise in endometrial thickness, and a gradual decrease in uterine artery pulsatility index (Bourne *et al.* 1996). However, the use of ultrasound is not suitable for home-use or for population based contraceptive studies.

1.11 Summary

Clinical research carried out to date suggests an urgent need for new methods of contraception and a need to explore the mechanisms of the existing contraceptives. Side effects such as unscheduled vaginal bleeding affect the acceptability of a contraceptive method and the detailed understanding of the mechanism of these side effects enables us to take appropriate steps to prevent them. The studies conducted as part of this Thesis aim to further the advancement in contraceptive research in such areas.

In order to explain the mechanism of post-coital contraceptive, we investigated the effect of LNG on the menstrual cycle and we also assessed the feasibility of using mifepristone as a once-a-month contraceptive pill.

Although compliance is a fundamental prerequisite for achieving the full potential efficacy of all drugs, including contraceptives, there is a dearth of information available on patient non-compliance with the use of different contraceptive methods. We sought to obtain insight into the compliant behaviour of women taking part in the contraceptive trial assessing the feasibility of administering once a month mifepristone.

The frequent and regular laboratory assays of steroid hormones in either blood or in urine have traditionally played a major role in research into female contraception. We

analysed the use of a personal fertility monitor as a reliable and more simple means to predict the potentially fertile period by monitoring frequently changing hormone levels.

To investigate the unexplained mechanism of mifepristone-induced endometrial shedding and vaginal bleeding, we evaluated the effects of mid-luteal phase administration of mifepristone on endometrial parameters.

CHAPTER 2

THE EFFECTS OF PERI-OVULATORY ADMINISTRATION OF LEVONORGESTREL ON THE MENSTRUAL CYCLE

2.1 Introduction

LNG 0.75 mg administered twice with the two doses 12 hours apart has been shown to be an effective method of EC when used within 72 hours of unprotected intercourse (Ho *et al.* 1993; WHO *et al.* 1998). Although the regimen is now licensed in the UK, USA and throughout much of Europe and is widely regarded as the EC method of choice (Guillebaud *et al.* 1998), the mechanism of action remains unknown. The mechanism of action of the Yuzpe regimen of emergency contraception (ethinyl estradiol 100 mcg and 0.5 mg LNG, two doses 12 hours apart, Yuzpee and Lancee 1977) is also incompletely understood but there is good evidence that it delays or inhibits ovulation in at least some cycles (Swahn *et al.* 1996; Croxatto *et al.* 2002). In the WHO study (1998) the efficacy of both LNG and the Yuzpe regimen decreased with time after intercourse (Piaggio *et al.* 1999) and both regimens had a similar effect on the timing of the subsequent menses, suggesting that the mechanism of action of the two regimens may be similar. It has also been shown that supra-physiological amounts of synthetic progestogens abolish the mid cycle LH surge leading to anovulation and delaying the onset of the subsequent menses (Kesseru *et al.* 1974; Landgren *et al.* 1990; Craft *et al.* 1975; Sopna *et al.* 1974; also refer Table 1.2).

To test the hypothesis that it acts as a post-coital agent by abolishing the pre-ovulatory LH surge and by delaying ovulation, we administered LNG 0.75 mg twice to 12 healthy female volunteers in the fertile period (immediately before ovulation) of the menstrual cycle and investigated the effects on the timing of ovulation and of the next menses; bleeding patterns; ovarian activity and LH concentrations.

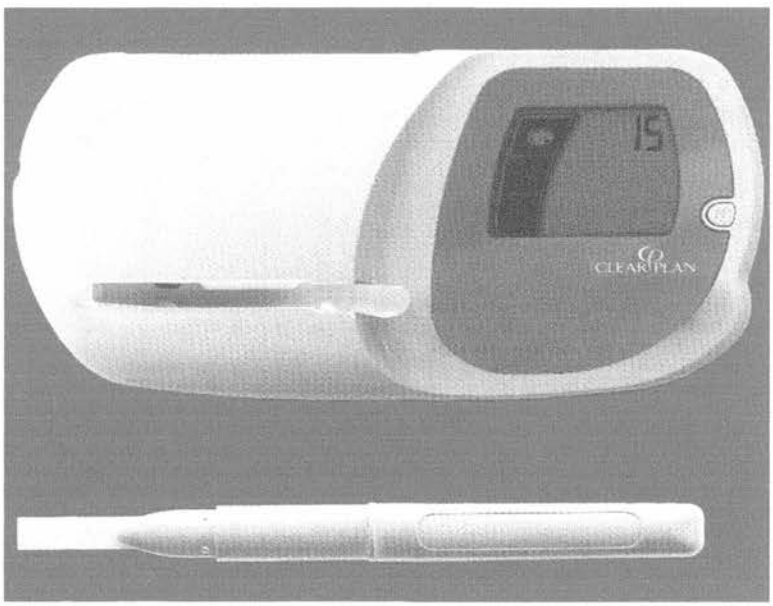
2.2 Materials & Methods

This was a prospective, randomised, double blind, cross-over study undertaken in one centre. Twelve healthy women (mean age 33 (range 26 - 41 years)) with regular cycles (mean 27.63 days (range 25 – 30 days) and mean BMI 25.71 (range 20 - 34)) were recruited. They were all using a reliable non-hormonal method of contraception or were abstinent during the study. All subjects gave written informed consent to

participation in the study, which was approved by the Lothian Research Ethics Committee.

A method was sought to provide a convenient means of identifying the fertile period prior to ovulation, and thereby to time the administration of LNG. A home-use fertility monitor was used for this purpose. This product is similar to the Personal System of Contraception (Persona, Unipath Bedford UK) (Bonnar *et al.* 1999) in that it monitors E3G and LH in urine, but differs in that it has been specifically designed for maximising the chance of conception. Unipath Diagnostics Co., Princeton, NJ, markets the fertility monitor in the US and Unipath Ltd., Bedford, UK markets it in Britain as ClearPlan Easy Fertility Monitor (CPEFM) (Figure 2.1).

Figure 2.1 *ClearPlan Easy Fertility Monitor with a urinary test stick* (LCD on the monitor shows peak fertility).



The system comprises a hand-held monitor and disposable dual-assay urine test sticks, and is used to simultaneously detect LH and E3G levels in early morning urine. The monitor optically measures the intensity of the lines that form on the test sticks after sampling, and the system will delineate three levels of fertility (Low, High and Peak fertility) according to the optical signal changes detected. Low Fertility will be displayed from day 1 of the menstrual cycle, until the hormone levels rise above the baseline levels. A change from Low to High Fertility is triggered by

detection of elevated E3G levels. The change from High to Peak Fertility is triggered by the detection of an LH surge. Peak fertility is displayed on the day of the LH surge and on the following day before returning to low fertility.

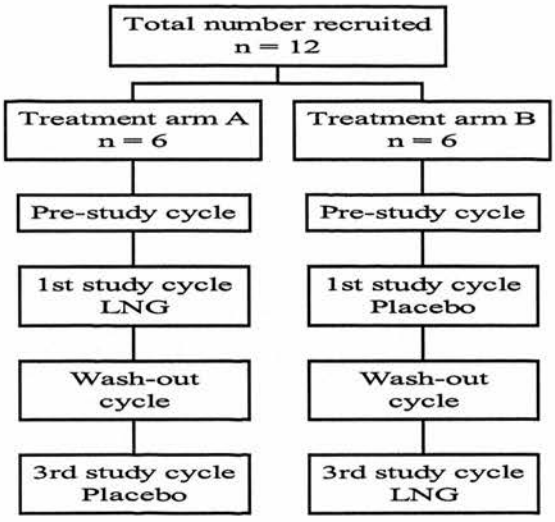
Each woman was studied during four cycles, and was issued with a monitor at the beginning of the study. Subjects were asked to use the CPEFM according to instructions, and familiarised themselves with the monitors by using it during a pre-study cycle to identify the days of high fertility and the day of the LH surge. They also recorded days of vaginal bleeding.

Data from the pre-study cycle was used to predict the timing of the LH surge and of the fertile phase during the study cycles and thereby to predict when treatment should be administered. The three study cycles followed immediately after the pre-study cycle. Six subjects were randomly assigned to treatment arm A and received LNG in the first study cycle and placebo in the third study cycle. The remaining six subjects were randomised to treatment arm B and received placebo in the first study cycle and LNG in the third study cycle. The second study cycle was a "washout" phase for all women during which time they also received placebo tablets (Figure 2.2).

The randomisation list was produced using SPSS Rv.Bernouilli function such that each study number was randomly assigned to either treatment arm A or treatment arm B with the same probability i.e. 0.5.

LNG and placebo were visually identical and were pre-packed. Each subject collected a sample of early morning urine daily from the first day of the first study cycle until and including the first day of the menstrual bleed signalling the end of the last study cycle. Samples were frozen and later assayed in batches (with all samples from one subject assayed in a single batch) for measurement of urinary LH, E3G and pregnanediol-3-glucuronide (P3G).

Figure 2.2 *Study design*



Quantitative assessment of urinary LH was performed using a LH MAIAclone kit (BIOSTAT-DIAGNOSTICS, Stockport, Cheshire, UK). This method incorporates two high affinity monoclonal antibodies into an immunoradiometric assay system and offers a working range of 1.5 – 200 mIU/ml. Urinary P3G was measured using a direct enzyme immunoassay sensitive (working range 0.16 ug/L – 20 ug/L), while direct immunoassay was used to measure E3G levels (working range 8.36 nmol/L - 2140 nmol/L). Intra-assay coefficients of variation were 6% for E3G, 10% for P3Gl and 3% for LH (Yong *et al.* 1992). Geometric means of daily replicates were divided by the respective daily creatinine concentration to correct for variations in the dilution of the urine specimen.

During study cycles 1 and 2 women were asked to take the study medication on the first day of high fertility as identified by the monitor. However, by the third study cycle, the variation in the number of High Fertile days (range 0-8 days) meant that the monitor could not be used to administer medication on LH-2 in every cycle. Therefore, we had to adopt a different method of calculating the anticipated day of the LH peak for each cycle based on the monitor information from the previous cycles (including the pre-study cycle). Hence, in the third study cycle, the medication was taken two days prior to the anticipated day of the LH peak. In all cycles the first tablet was taken at 11.00 hours and the second at 23.00 hours. A sample of venous blood was collected 5 – 7 days after treatment, stored and later assayed for

progesterone using Coat-A-Cont solid-phase radio-immunoassay. The subjects kept a daily record of all vaginal bleeding experienced during the 4 cycles, the fertility status information displayed each day on the monitor LCD and the days on which the study medications were taken.

2.2.3 Statistical Analysis

We calculated that a total of six subjects in each of the two treatment arms would give more than 90% power to detect a delay of menses of $\geq$ 5 days in 95% of cycles. The sample size calculation indicated that only 2 subjects per treatment order arm are required for 90% power if we assumed that LNG delayed ($\geq$ 5 days) the subsequent menses in 95% of cycles and the menses would be delayed in 5% of cycles after placebo administration. Since greater numbers were required for the reliability of estimates and to take account of the fact that the menstrual cycles of subjects may have been less regular during the study than had been indicated at the recruitment stage, we recruited 12 subjects.

Preliminary analysis was performed to determine whether parametric tests were appropriate for analysis of the data. Outlying data points were investigated while the treatment was still blinded. The period effect and interaction between treatment and period effect were tested (two sample t-test) before progressing to testing of a treatment effect and was non-significant. Comparisons between LNG versus placebo cycles, was tested by paired t-test. Since the data appeared to be skewed a non-parametric Mann-Whitney U test was performed to compare the difference between urinary E3G levels on the day of LNG treatment in cycles that had delayed LH peak and the 8 cycles that did not.

For the purpose of the study the following definitions based on the quantitative data were created.

A significant delay in the onset of next menses: Delay of 5 or more days from the expected onset of menses (based on the mean cycle length for the 2 placebo cycles).

The LH peak was defined as a significant rise in urinary LH concentration, with a minimum of 50% rise above the average baseline level for 4 preceding days and which remained elevated for a minimum of 3 days.

The first day of the LH peak was defined as the day of the first significant rise (>50% above the baseline) seen at the beginning of the LH peak.

Retrospectively predicted first day of the LH peak for the treatment cycles was the calculated mean of the first day of LH peak in the two placebo cycles.

A significant delay in the first day of the LH peak: Delay of 5 or more days from the expected first day of the LH peak in the treatment cycle (based on the mean first day of the LH peak during the 2 placebo cycles).

Follicular phase: time from the first day of the menses until the day of the first significant rise in urinary LH (LH+0) inclusive.

Luteal phase: time from the day after the first day of the urinary LH peak (LH+1) until, and including, the day before the first day of the next menses.

2.4 Results

A total of 48 menstrual cycles were studied - 12 pre-study cycles and 36 study cycles. Data from daily urine samples were available for 34 out of the 36 study cycles. In one woman (S103) the first study cycle, which was a placebo cycle, was prolonged (41 days) as a consequence of a delay in ovulation. Her usual cycle length was 25 days, this cycle was excluded from the analysis. In a second woman (S110) there were no daily urine samples available from the washout cycle as she was abroad on holiday. Therefore, daily urine samples were only available for this subject from two study cycles (the treatment cycle and one placebo cycle).

2.4.1 Timing of administration of LNG

Six women took LNG in the first study cycle and six took it during the third study cycle. During the first study cycle, 10 women took the tablet (either placebo or LNG) on the first day of high fertility as indicated by the monitor. The remaining two women took the tablet on the first day of the urinary LH peak because the monitor failed to identify any High fertile days prior to the LH peak.

The variation in the number of High fertile days (0-8 days) declared by the monitor meant that the system could not be used to predict LH-2 in every cycle. Therefore for cycle three we calculated the anticipated first day of the urinary LH peak from the information gathered from the pre-study cycle and study cycles 1 and 2 for each woman and instructed subjects to take the tablet two days before the anticipated day of the LH peak.

After completion of the study, we retrospectively calculated the predicted first day of the urinary LH peak for every treatment cycle based on the mean of the first day of LH peak in the two placebo cycles and the pre-study cycle. When we applied this retrospectively predicted definition to all 12 treatment cycles, the day of taking LNG ranged from six days before until one day after the first day of the anticipated LH peak. However, in reality, LNG was never taken after the first significant rise in urinary LH concentrations in any treatment cycle. The timing of the LH peak in each of the four cycles, the predicted day of the LH peak day and the timing of LNG and placebo treatment in relation to the start of the actual LH peak are shown in table 2.1.

2.4.2 Cycle length

Treatment with LNG in the pre-ovulatory period significantly prolonged (by 5 days or more) the mean cycle length in four women (33 % of the sample, Table 2.2). All four women reported vaginal spotting two to three days after taking LNG and they all had a second episode of vaginal bleeding between 9 and 16 days after the delayed LH peak. In the remaining eight women there was no significant difference in cycle length between treatment and placebo cycles. One of this group of women however, reported light vaginal bleeding starting a week after taking LNG, the bleeding continued until she started what she regarded as a normal menstrual period which followed a fall in urinary pregnanediol levels. Her hormone profile during the treatment cycle follows a normal pattern. The four women, who had prolonged cycles, took LNG on a day when urinary E3G was at a low level when compared to the remaining 8 women but this difference did not reach significance (8 vs. 19.16 mmol/mol, $p = 0.12$).

Table 2.1. *Timing of LH peak, predicted day of the LH peak and timing of LNG in treatment cycles for all subjects*

Subjects	Day of the LH peak (LH >50%)				Predicted day of the LH peak In the treatment cycle	Timing of LNG in relation to the day of LH peak in the treatment cycle	Timing of LNG in relation to the predicted day of the LH peak
	PSC	Placebo	Placebo (washout)	Treatment			
S101	12	13	9	10	11	-1	-2
S102	*M*	20	19	21	19	-1	+1
S103	12	*27***	9	11	9	-1	+0.5
S104	*M*	11	12	12	11.5	0	-2
S107	17	14	14	13	14	-1	-2
S111	12	11	15	12	13	-1	-3.5
S112	11	11	12	13	11.5	-5	-2
S105++	14	13	15	14	14	-2	-3.5
S106*	14	14	12	23	13	-11	-1
S108*	17	18	18	25	14	-13	-2
S109*	14	10	10	38	10.5	-31	-3.5
S110*	16	14	££	40	18	-25	3

++ The woman with normal LH peak but no significant rise in pregnanediol in the luteal phase = anovulatory cycle.

M= LH peak missed by the CPFM

***= excluded, unusually delayed ovulation*

£££ = excluded, no daily urine available

** Women who had a delay of the LH peak by >5days.*

Table 2.2 *Mean length of placebo and treatment cycles*

Mean cycle length	Treatment cycles	Placebo cycles
All cycles n = 12[1]	32.17 (SD +/-3.36)	26.33 (SD +/-.42)
N = 4, Delay of > 5days[2]	42.75 (SD +/-8.42)	27.13 (SD +/-1.84)
N = 8, remaining cycles[3]	24.88 (SD +/-2.1)	26.13 (SD +/-1.69)

1. p = 0.14, 2. p = 0.04, 3. p = 0.12

2.4.3 The first day of the LH peak.

In the four women with long cycles, LNG appeared to abort the LH peak and a subsequent LH peak occurred 7 to 16 days later followed by a normal rise in urinary pregnanediol. The urinary hormone profile of one-woman (S110) is illustrated in charts 2.1a and 2.1b. In the remaining eight women, LNG did not affect the timing of the LH peak when taken immediately before ovulation. Chart 2.3 illustrates the hormone profile in one of these women (S101).

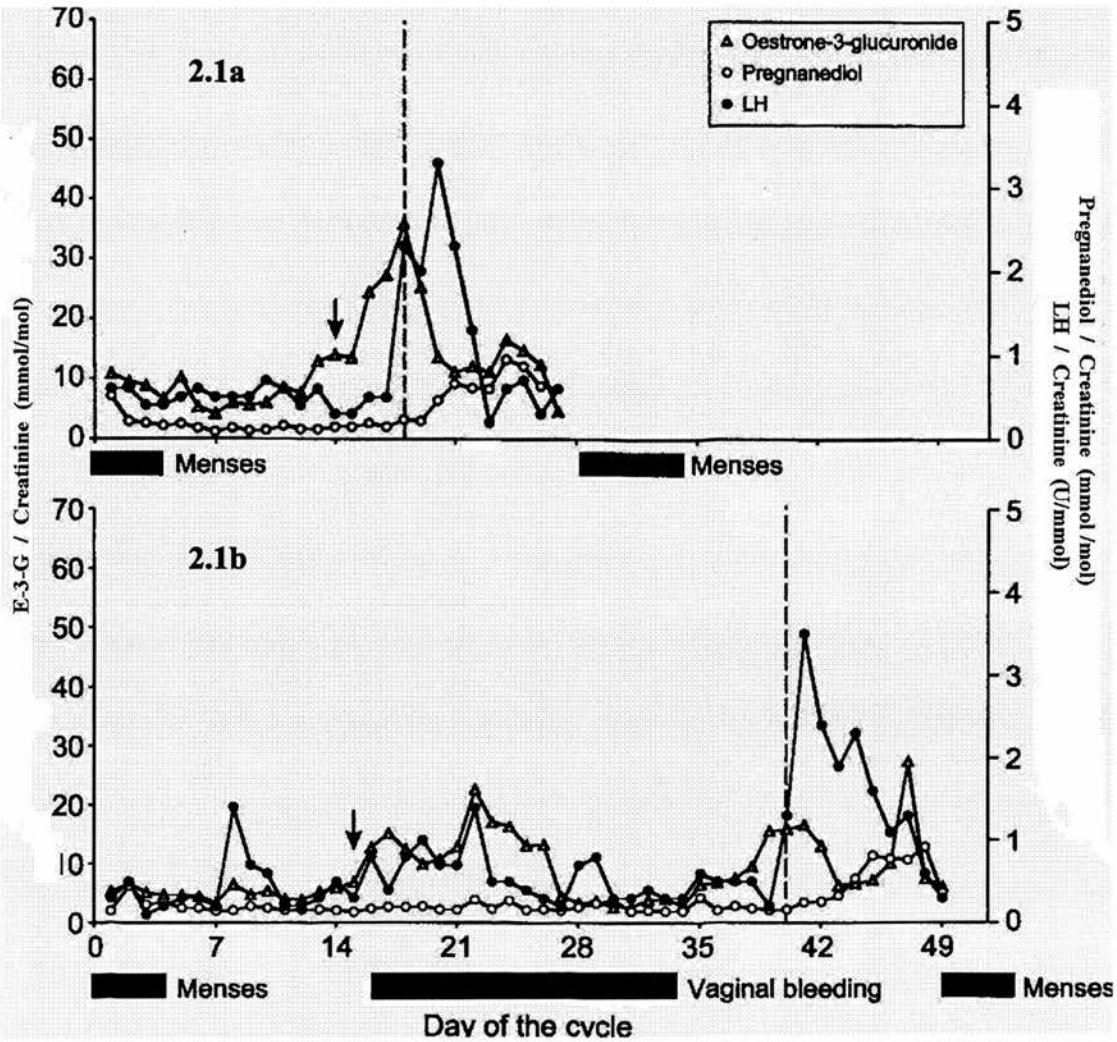
Charts 2.1a & 2.1b

Daily levels of LH (●), E3G (Δ), and pregnanediol (O), in urine relative to the cycle day.

2.1a Placebo cycle of a woman (S110).

2.1b Treatment cycle of the same woman (S110) showing significantly prolonged cycle following pre-ovulatory LNG.

↓ Day of taking LNG or placebo tablet ····· Day of the LH surge



Charts 2.2 and 2.3

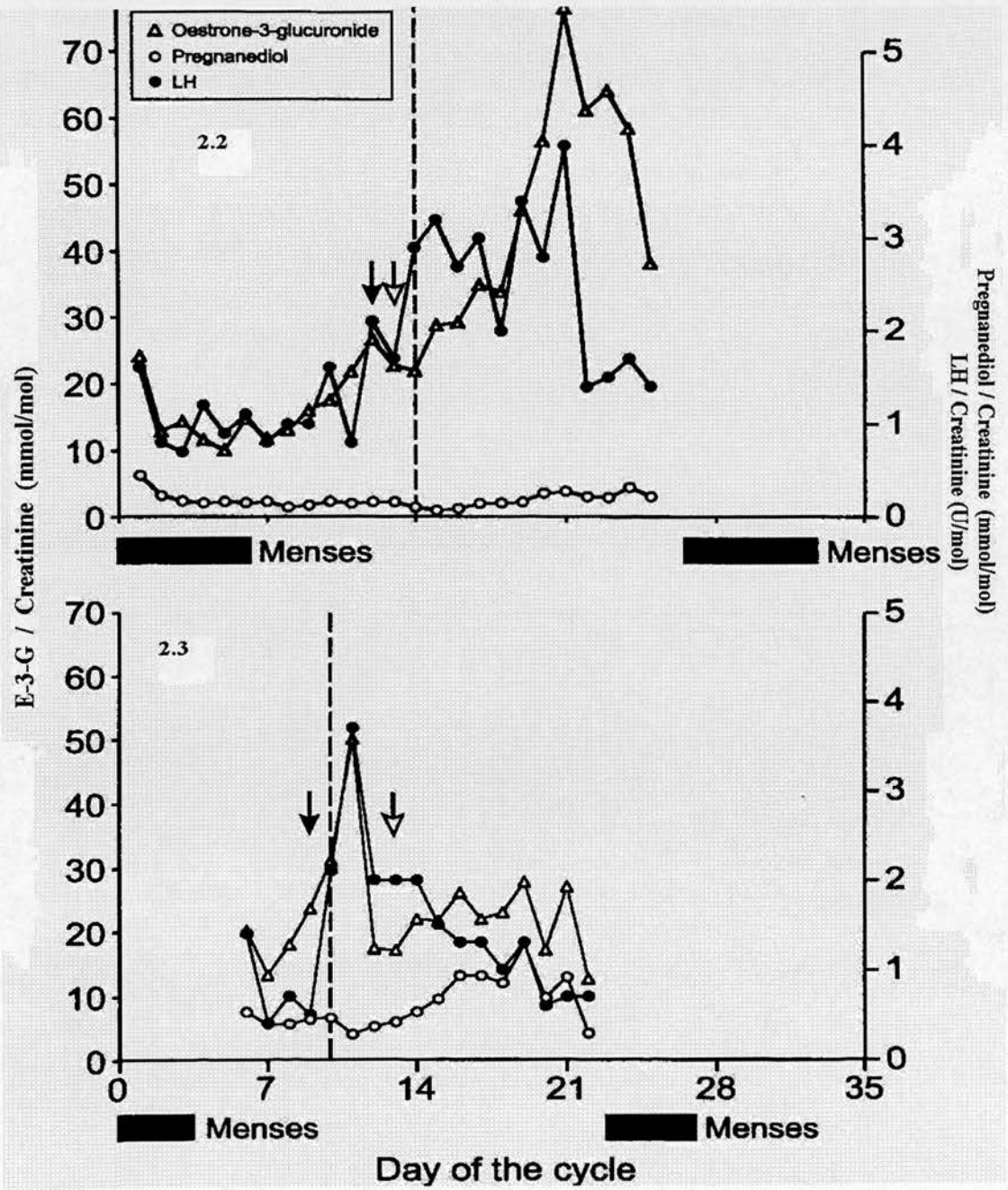
Daily levels of LH (●) E3G (Δ), and pregnanediol (O), in urine during the treatment cycles,

2.2 Of the woman (S105) with no significant rise in pregnanediol following LNG

2.3 Of a women (S101) with apparently normal cycle length

↓ Day of taking LNG Day of the ⋮▼ LH surge in the placebo cycle

..... Day of the LH surge



2.4.4 Length of the luteal phase

In all twelve volunteers the luteal phase was significantly shortened following treatment with LNG as compared with the placebo cycles (mean length 11.5 days (SD +/-1.8) vs. 12.9 days (SD +/-2.5) p = 0.005, Table 2.3).

Table 2.3 Length of the luteal phase (n = 12)

Patient	Treatment cycle	Mean for Placebo cycles
101	12	13.5
102	8	8.5
103	14	14
104	13	15.5
107	13	14
111	12	13.5
112	10	14
105 ++	11	13.5
106 *	12	12
108 *	10	11.5
109 *	13	16.5
110 *	9	9

++ The woman with normal LH peak but no significant rise in pregnanediol in the luteal phase = anovulatory cycle.
** Women who had a delay of the LH peak by >5days.*

2.4.5 The effect on total LH during the luteal phase

Daily urinary LH concentrations were summated from the first day of the LH peak (LH +0) up to the day before the first day of the next menses to give a value for total LH concentrations. The eight women in whom pre-ovulatory LNG did not affect the cycle length, showed a significant (p = 0.01) decrease in total LH in the treatment cycles (18.66 U/mol creatinine (SD +/-8.9)) as compared with the placebo cycles (27.08 U/mol creatinine (SD +/-13.57)). In contrast among the four women with significantly longer cycles after taking LNG, there was no difference in the total LH secretion in the luteal phase (mean total LH = 18.83 U/mol creatinine (SD +/-8.3)

versus mean total LH for placebo cycles of 17.75 U/mol creatinine (SD +/-2.8) p = 0.81).

2.4.6 Effect on pregnanediol in the luteal phase

Comparing the sum of daily pregnanediol concentrations in the luteal phase (from LH +1 onwards up to the day before the first day of next menses) during the treatment and placebo cycles was employed to indirectly assess corpus luteal function.

In one woman (S105) after taking LNG, there was no significant rise in urinary pregnanediol levels (>0.5 mmol/mol creatinine as expected in the mid-luteal phase) despite an apparently normal LH peak. The mid-luteal serum progesterone level during this treatment cycle was consistent with an anovulation (<5 nmol/L) (chart 2.2).

In the remaining 11 subjects the total values of pregnanediol did not show a significant difference between placebo or treatment cycles.

2.5 Discussion

Evaluation of daily hormone concentrations confirmed that all twelve women in our study took LNG before the LH peak, and before ovulation.

Seven women had apparently normal ovulatory cycles after taking LNG. Five of them took LNG on the day before the LH surge and one on the day of the surge. It is possible that the timing of LNG in these women was "too late" to influence an event already well underway. Recently, others have shown a similar effect when LNG is taken immediately prior to the LH surge, late in the follicular phase (Durand *et al.* 2001).

In four women (25%), LNG aborted the LH peak, delayed the ovulation and lengthened the cycle. All four women took LNG 2 to 4 days before the predicted LH

surge (based on the two placebo cycles). One woman did not ovulate at all despite having an LH surge 2 days after taking LNG.

It is apparent that different women respond differently to the administration of LNG. The effects observed may be related to administering LNG at a specific stage in follicular development. Even though the timing of the LNG in relationship to the onset of the LH surge did not appear to be different between women in whom ovulation was affected and those in whom it was not, it would be naïve to accept that our predicted day of the LH peak based on information gathered in two cycles was always accurate. In fact, the four women with delayed ovulation had apparently low urinary E3G on the day the treatment was administered. This suggests that they took LNG at an earlier stage of follicular maturation and a similar effect was not seen in the others who took LNG at a more advanced stage of follicular development. A more detailed study by Duran *et al.* also suggested that LNG in mid follicular phase suppress follicular maturation and secretion of E2 and P4, and thereby inhibit LH surge and ovulation (Duran *et al.* 2001). When administered immediately prior to the onset of the LH surge, LNG did not affect the LH surge, ovulation or the cycle length in their study. They employed daily serum levels of gonadotrophins and steroid hormones and ultrasound scans to correlate follicular size and maturity to the time the administration of LNG. In agreement with this explanation, another recent study has also shown that when LNG was administered 2 days before the anticipated LH surge, the ovulation was not affected (Marions *et al.* 2002).

The available literature suggests a direct suppression of pre-ovulatory E2 rise by progestins (Schreiber *et al.* 1980; Goodman *et al.* 1982; Fortune *et al.* 1983; Weibe *et al.* 1984) and that may be the mechanism by which LNG inhibits the LH surge.

Many have reported a shorter luteal phase, after peri-ovulatory administration of LNG (Johansson *et al.* 1971; Sopna *et al.* 1975; Craft *et al.* 1975; Landgren *et al.* 1989; Duran 2001). In our study, the seven women who apparently ovulated normally had a reduced total luteal LH and a shortened luteal phase. Basal levels of LH are

essential for the normal secretory function of the corpus luteum (Vande Wiele *et al.* 1970). In the mid-luteal phase LH inhibition by the administration of GnRH antagonists consistently resulted in luteolysis in women as well as in non-human primates (Hutchison *et al.* 1984; Hall *et al.* 1991; Ravindranath *et al.* 1992). There are no direct ways of measuring the corpus luteal function. Although we did not find a significant difference in the urinary pregnanediol levels after LNG, it is possible that the shortened luteal phase observed was a consequence of reduced total LH and may have a contra-gestive effect.

If LNG acts as an emergency contraceptive only by interfering with ovulation, the expected efficacy should fall below 42% (5 out of 12 women). Ho and colleagues reported that LNG reduced approximately 60% of the expected number of pregnancies (estimates were based on the table of probabilities of pregnancy at different cycle days by Dixon *et al.* 1980). LNG faired better in the WHO study (WHO 1998) with overall 85% reduction of expected number of pregnancies (the analysis of the prevented fraction was based on the modified Wilcox estimates of conception probabilities (Trussell *et al.* 1998)).

Both studies reported effectiveness against estimates based on historical data. The fertile period was determined on the assumption that ovulation occurred 14 days before the next expected menses. The validity of using these estimates directly relies on the accuracy of reported menstrual cycle data. Women do not always record their menses, and the sexual intercourse responsible for requesting EC maybe unpremeditated. Reporting errors are common and the estimates can be inaccurate. In addition, other factors such as biological variability of the day of the ovulation and the length of the luteal phase, factors affecting the probability of pregnancy unrelated to the timing of intercourse, and heterogeneity among couples in fecundability can distort the estimated number of pregnancies. In a study comparing the efficacy of the Yuzpe regimen of EC with a single dose of mifepristone (Glasier *et al.* 1992), there were frequent discrepancies among subjects between the stage of the cycle as estimated from the date of the LMP and that suggested by circulating concentrations

of progesterone. In a recent report, Sterling & Glasier went on to confirm their previous suggestions, and they showed that the date of LMP, timing of intercourse, and the number of acts of intercourse in the same cycle were inaccurately recalled by a significant proportion of women requesting EC (Sterling & Glasier 2002). There has never been a placebo-controlled trial of EC. Thus, it is possible that the genuine effectiveness of LNG as an EC is less than 42%.

On the other hand, the discrepancy noted in the estimated effectiveness of LNG and the prevalence of ovulation delay or inhibition in our study may be due to mechanisms of action other than interference with ovulation. Our study was not designed to investigate the other possible mechanisms by which LNG works. However, one woman in our study reported slight vaginal bleeding after taking LNG with an apparently normal LH peak, cycle length and hormone profile. This may suggest an additional effect of LNG on the endometrium. Landgren and colleagues (Landgren *et al.* 1989) showed that follicular phase administration of multiple doses of 0.75 mg LNG suppressed the proliferative activity of the endometrium, while no significant endometrial effect was seen if it was taken in the luteal phase. Glandular atrophy, stromal decidualisation and epithelial cell inactivation has been observed after chronic local exposure of the endometrium to LNG (Pakarinen *et al.* 1995). Locally administered LNG also induces expression of the potent contraceptive glycoprotein Glycodelin A during the implantation period (Mandelin *et al.* 1997). In high doses (20 – 40 ng/ml) progesterone inhibits the growth of human capillary endothelial cells in culture (Peek *et al.* 1995). Therefore, the question remains as to whether similar alterations occur in the endometrium after taking the EC regimen of LNG, and whether these changes are sufficient to prevent implantation and account for the observed contraceptive efficacy of LNG. The two recent studies, which examined the effect of peri-ovulatory phase administration of LNG on endometrium, did not report any significant morphological changes (Duran *et al.* 2001; Marions *et al.* 2002).

The effect of progestogens on cervical mucus and on the cervix is well documented and this is thought to be the main mechanism by which the progestogen-only pill exerts its anti-fertility action (Odeblad *et al.* 1972; Daunter *et al.* 1976; Moghissi *et al.* 1973). However, even if LNG has an effect on cervical mucus, which interferes with sperm penetration, that action is unlikely to prevent pregnancy when taken some 12 - 72 hours after coitus.

Progesterone stimulates a rapid influx of calcium into the spermatozoon via a non-genomic action (Tesarik *et al.* 1996; Aitken *et al.* 1996; Blackmore *et al.* 1996) and induces an acrosomal reaction (Oehninger *et al.* 1994). An impaired response of the spermatozoon to progesterone is a clinically well-recognised defect in the infertile male (Falsetti *et al.* 1993). Given their total lack of transcriptional ability, it would appear that in the sperm the non-genomic progesterone receptor would have to be responsible if LNG were to have an EC effect.

One woman (S103) in our study showed a delayed LH peak (on day 27) during a placebo cycle and subsequently the length of that cycle was prolonged to 41 days (her usual cycle length was 25 days). In contrast to the four women who experienced similar prolongation of the cycles after taking LNG, this woman (S103) did not report any inter-menstrual vaginal bleeding. Although we excluded this cycle from our analysis, similar spontaneously occurring prolonged cycles (with delayed ovulation) can influence the results of studies into EC.

The fertility monitor is a product designed to indicate the potentially fertile days leading up to ovulation, through the detection of a significant rise in the levels of the urinary oestrogen metabolite E3G. The position of this rise relative to the time of ovulation will vary between women, and therefore the monitor will not consistently provide two days warning of the LH surge in every cycle. It was understood that no home use method could consistently provide such focussed warning of ovulation, but the monitor was considered to represent the best available home use technology for ovulation prediction.

The reason for using the monitor to time the administration of LNG or placebo also to avoid having to subject the volunteers to regular blood samples and ultrasound scans.

However, due to the variability in the number of high fertile days declared prior to the LH surge, greater reliance had to be placed on calendar calculations to predict the LH surge.

In conclusion, we suggest that LNG taken immediately before ovulation acts as an EC by delaying or preventing ovulation. Other plausible actions of LNG including the retardation of the endometrium, interfering with sperm motility and altering cervical mucus may be important, and need to be explored further.

CHAPTER 3

FEASIBILITY OF ADMINISTERING MIFEPRISTONE AS A ONCE-A-MONTH CONTRACEPTIVE PILL

3.1 Introduction

While hormonal contraception is extremely popular (it is used by almost 100 million women world-wide) many women continue to be deterred from using it because of perceived risks to health such as breast cancer and due to side effects such as weight gain. Most of the risks and the side effects are the results of prolonged exposure to steroids and many women, in a variety of cultural settings, find the idea of a pill, which they need take only once each month, an attractive concept (Rimmer *et al.* 1992; Glasier *et al.* 1999).

Progesterone is essential for the establishment and maintenance of human pregnancy. The anti-progesterone mifepristone is a synthetic 19-norsteroid, which acts by blocking the action of progesterone at the receptor level (Spitz & Bardin 1993), and thus, having multiple potential anti-fertility actions. When administered in the early luteal phase mifepristone retards endometrial development, without disturbing the timing of menses (Swahn *et al.* 1988; Berthois *et al.* 1991; Mentausta *et al.* 1993). It also changes uterine contractility to a pattern more usually seen in the late luteal phase (Gemzell-Danielsson *et al.* 1990). In 1993 Gemzell-Danielsson and colleagues conducted a pilot study in which a single dose of 200 mg of mifepristone was given in the early luteal phase (2 days following the peak of the LH in urine). Out of 124 cycles in which coitus took place during the fertile period, they observed only 1 pregnancy. There was no disruption of the timing of the subsequent menstrual bleed, although in 32% of the cycles slight vaginal bleeding was reported 2 – 3 days after treatment.

The main problem in developing a once-a-month contraceptive is finding a method that both reliably and easily identifies the start of the LH peak. Gemzell-Danielsson tried to solve this problem by using the LH sticks for home urine testing (Ovu-quick, Organon). In their study 12 out of 169 cycles were deemed to be anovulatory. However, it is not possible to determine if the LH peak truly was absent, or if the method failed to detect a surge. The woman may have read the test result wrongly or even forgot to perform a test

on the appropriate day. Moreover, anovulatory cycles would not be expected to exceed 5 per cent among healthy women in that age group (18 - 35 years) (Vollman *et al.* 1977).

Unipath (Bedford, UK) have developed a Fertility Awareness Monitor, which simultaneously detects LH and E3G in a sample of early morning urine. The monitor utilises the information from both of these parameters to determine the day of the LH peak. The time from first significant rise of LH in the urine to ovulation is reported to be around 24 - 48 hours (Collins *et al.* 1996). The monitor thus, should provide a convenient method of identifying the early luteal phase. In addition, it provides a 6-month record of the actual signal levels corresponding to daily hormone levels in urine, and of the days on which tests were performed.

We investigated the use of the monitor to identify the LH surge in a group of women who used 200 mg of mifepristone on day LH +2 as their sole method of contraception.

3.2 Subjects and Methods

3.2.1 Subjects

This was a single centre study in healthy female volunteers, approved by the Lothian research ethics committee. All subjects gave written informed consent to participation. Fifty two sexually active women, with regular (25 – 32 day) menstrual cycles were recruited from a large Family Planning Clinic in Edinburgh. If any women had a significant medical condition or if they or their partners had a history of fertility problems, they were excluded from the study.

3.2.1.1 Treatment group

Thirty two women were recruited to the treatment group. None had been taking hormonal preparations within the 2 months prior to the start of the study and all had at least 2 spontaneous menstrual periods since stopping hormonal contraception. All women underwent screening at the time of recruitment including routine physical and

gynaecological examination. A venous blood sample was taken for full blood count, serum biochemistry and liver function. The study period was from day 1 of the menstrual period following screening, and lasted for up to seven consecutive menstrual cycles in which subjects took 200 mg mifepristone once per month.

3.2.1.2 Control group

The control group consisted of 20 healthy women with regular menstrual cycles who were trying to become pregnant (for less than 6 months prior to the enrolment into the study) and hence, were not using contraception. They were provided with a monitor, which they used according to the manufacturer's instructions. Women were advised that their chance of conception would be higher if they were to have sexual intercourse during the fertile period, identified by the monitor. The controls took part in the study until pregnancy occurred or for a maximum of 6 cycles if they did not conceive.

3.2.2 Method

All subjects and controls were provided with a home use hormone monitoring system (Unipath, Bedford, UK). The system comprises a hand-held monitor and disposable dual-assay urine test sticks, and is used to simultaneously detect LH and E3G levels in early morning urine (Figure 2.1). The monitor optically measures the intensity of the lines that form on the test sticks after sampling, and the system will delineate three levels of fertility (Low, High and Peak fertility) according to the optical signal changes detected. Low fertility will be displayed from day 1 of the cycle, until the hormone levels rise above the baseline levels. A change from Low to High fertility is triggered by detection of elevated E3G levels. The change from High to Peak fertility is triggered by the detection of an LH surge.

Peak fertility is displayed on the day of the LH surge and on the following day. Subsequently High fertility is displayed for one day prior to a return to Low fertility. At the start of each menses, the subjects pressed the 'm' button on their monitor to initiate

that cycle of use, at a time suitable for testing the first urine of the day. For the rest of the month, the subjects were required to consult the monitor display each morning (3 hours either side of the time when 'm' button was set) to determine whether they needed to perform a test that day. Outwith this six hour time window the monitor would not accept a test. The system requests one test every day for up to a total of 10 or 20 tests, depending on the length of the woman's cycle, and the timing of her LH surge. Embedded software within the monitor collects and analyses data from each cycle to identify and display fertility status to the user, and stores data for several months (Figure 3.2 & 3.3). This product is similar to the Personal System of Contraception (Persona, Unipath Bedford UK) (Bonnar *et al.* 1999) in that it monitors E3G and LH in urine, but differs in that it has been specifically designed for maximising the chance of conception. Unipath Diagnostics Co., Princeton, NJ, markets the fertility monitor in the US and Unipath Ltd., Bedford, UK markets it in Britain as Clearplan Easy Fertility Monitor (CPEFM) (Figure 2.1).

Mifepristone was taken two days after the day of the first day of Peak Fertility (LH surge). With each cycle, subjects followed the same protocol, and were reviewed by the investigator monthly, on day LH +2. Just before taking the 200 mg tablet of mifepristone, a venous blood sample was taken, and later assayed for progesterone. At the beginning of the study, if the LH surge was not identified by day 21 of the cycle, the subject was instructed to continue testing, but mifepristone was not given in that cycle. The subject was also advised to use barrier contraception from day 21 until the onset of the next menses. After the second pregnancy (which occurred due to a failure in detecting an LH surge), we changed this practice. We calculated the estimated day of LH surge for each month based on information from the previous cycles. If the women did not detect an LH surge either within 3 days after the anticipated day of LH surge or by day 19, a blood sample was taken for rapid serum progesterone assay. If the progesterone level was >5nmol/L and if the woman was at risk of pregnancy, mifepristone was administered. All subjects and controls kept a menstrual record card,

recording all vaginal bleeding experienced during the study and the days on which they had sexual intercourse.

Figure 3.2 *Information retrieved from the Fertility monitor-1*

This figure illustrates the monitor display as seen by the volunteer each day.
Empty box = low fertility, Half filled box = high fertility,
Completely filled box + egg = peak fertility.
Underneath each box, a solid line represents a correctly performed test while a missed / inaccurately performed test is represented by a broken line. Volunteer using this particular monitor showed perfect compliance.

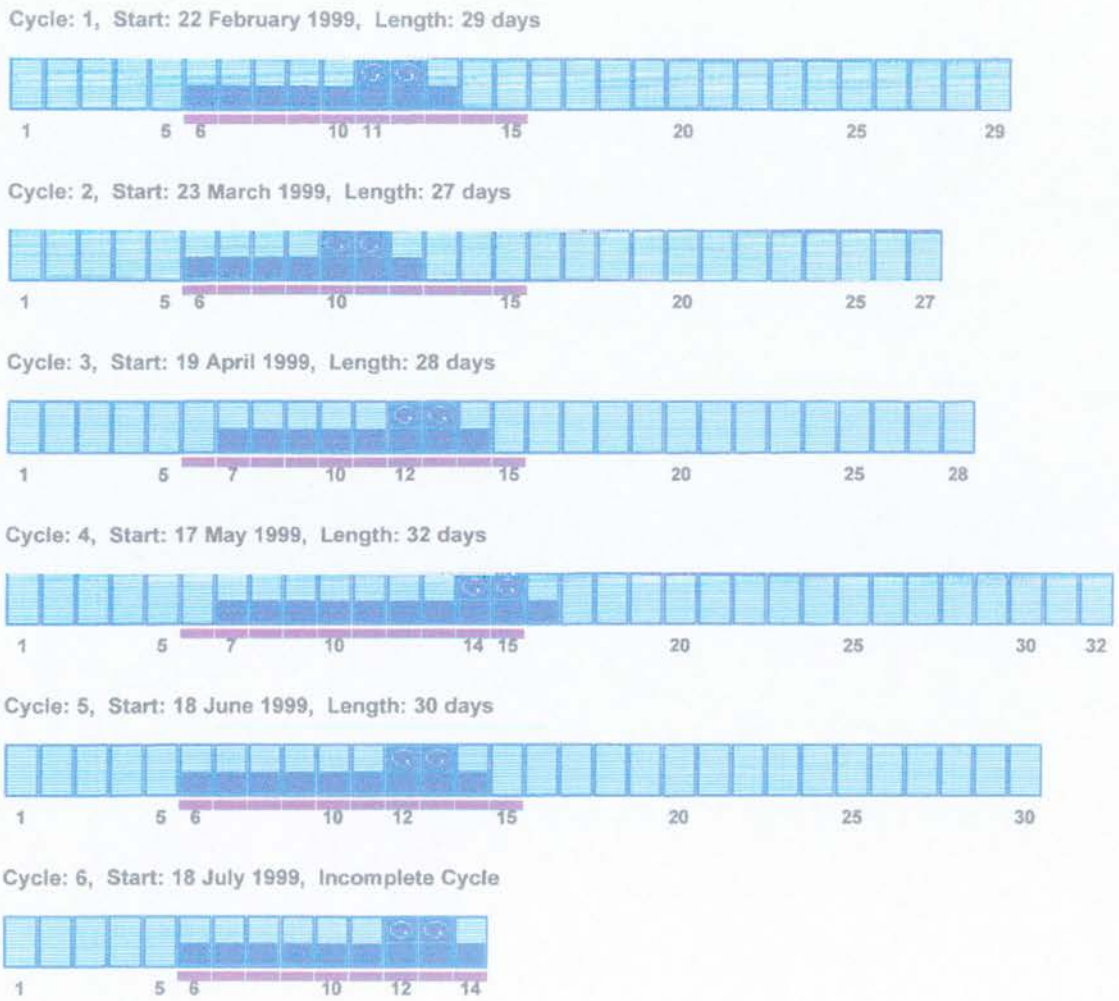
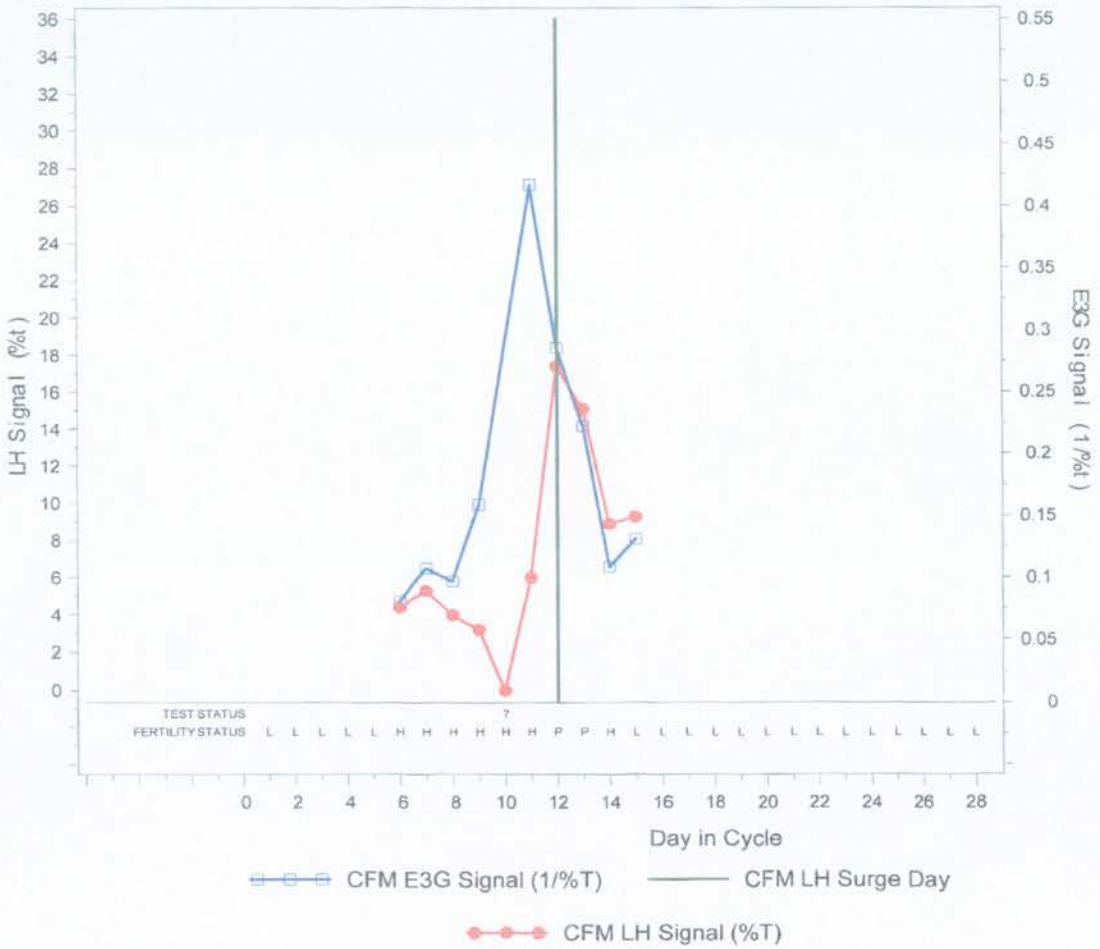


Figure 3.3 *Information retrieved from the Fertility monitor-II*

This figure illustrates the additional information that can be stored and retrieved from the monitor on the details of each test.
The closed circles represent the LH signal, and the open squares represent the E3G signal. The solid green line indicates the LH peak. Fertility status is indicated as L = low fertility, H = high fertility, & P = peak fertility.



Subjects also marked the first day of the Peak fertility as identified by the monitor and the day of taking the study medication.

If menstruation was overdue by more than one week the investigator performed a pregnancy test. Provided this was negative, the subject continued in the study and the next cycle was deemed to start with the onset of menses. Since the effect of mifepristone

taken in very early pregnancy is unknown, and teratogenic effects could not be ruled out, women who would not consider terminating any pregnancy were not recruited to the treatment group.

At the end of the study, the subjects attended for a final visit, when a routine physical and gynaecological examination was performed. Full blood count, serum biochemistry and liver function were reassessed.

The following definitions were created for the purpose of the study.

Imperfect Use was defined as failure to detect an LH surge through performing the test incorrectly (e.g.; dipping a test stick in urine 30 or more minutes before it being read by the monitor), or failing to perform tests as requested by the monitor.

Monitor method failures were defined as failure to detect an LH surge despite performing all tests as requested.

High fertile days: days preceding the urinary LH surge as indicated by the monitor to be potentially fertile.

Peak fertile days: The first day of a significant rise in urinary LH detected by the monitor, and the following day.

The Fertile period of the cycle was defined as three days before until two days after the urinary LH surge (LH -3 to LH +2).

Exposure cycles were cycles in which women reported having sexual intercourse at least once during the fertile period.

3.2.3 Statistical analysis

Cycle lengths and serum progesterone concentrations were compared by two-sample t-tests. Confidence limits for efficacy were derived from confidence limits for relative risk calculated by the Greenland and Robins method (Greenland & Robins 1985).

3.3 Results

Table 3.1 shows the demographic characteristics of the women who took part in the study. The women in treatment group were slightly younger (mean age 30 years) than those in the control group (mean age 32.92 years). Otherwise there were no differences between subjects and controls.

Table 3.1 Demographic data

		Treatment Group N = 32	Control Group N = 20
Age	Range	18 – 39	26 – 40
	Mean (+/- SD)	30 (+/-5.4)	32.9 (+/-4.5)
BMI	Range	19 – 38	21 – 29
	Mean (+/- SD)	23.6 (+/-4.3)	23.8 (+/-2.7)
Smokers	(%)	7 (21.9)	1 (5)
Non-smokers	(%)	21 (65.6)	16 (80)
Ex-smokers	(%)	4 (12.5)	3 (15)
Previous pregnancies	1+ (%)	19 (59.4)	14 (70)
	never been pregnant (%)	13 (40.6)	6 (30)
Ever abortion (%)		15 (46.9)	5 (25)
Married / Co-habiting	(%)	28 (87.5)	20 (100)
Single (with a regular boy friend)	(%)	4 (12.5)	0 (0)

3.3.1 The probability of pregnancy in the control group

20 women were recruited to the control group and three withdrew before completing the study. Two withdrew from the study as they found using the system "too stressful" and one withdrew because she no longer wished to plan a pregnancy. Data were collected from 50 control cycles during which 12 pregnancies occurred. Average frequency of intercourse was 1.7 episodes per week in the 39 control cycles in which the women kept a record of their sexual activity. In 37 cycles women had intercourse at least once during

the fertile period. In two cycles intercourse did not occur during the fertile period, while in 11 cycles the exposure status was unknown, as women failed to keep a record of sexual activity. Eight pregnancies occurred in the first exposure cycle.

If we assume that all 11 cycles from which information on sexual activity was lacking were exposure cycles, the probability of pregnancy was 0.25. However if those cycles were all non-exposure cycles, the probability of conception would be 0.32. Therefore among the control group the overall probability of pregnancy if sexual intercourse took place at least once during the fertile period lies between 0.25 – 0.32.

3.3.2 Contraceptive efficacy of the method

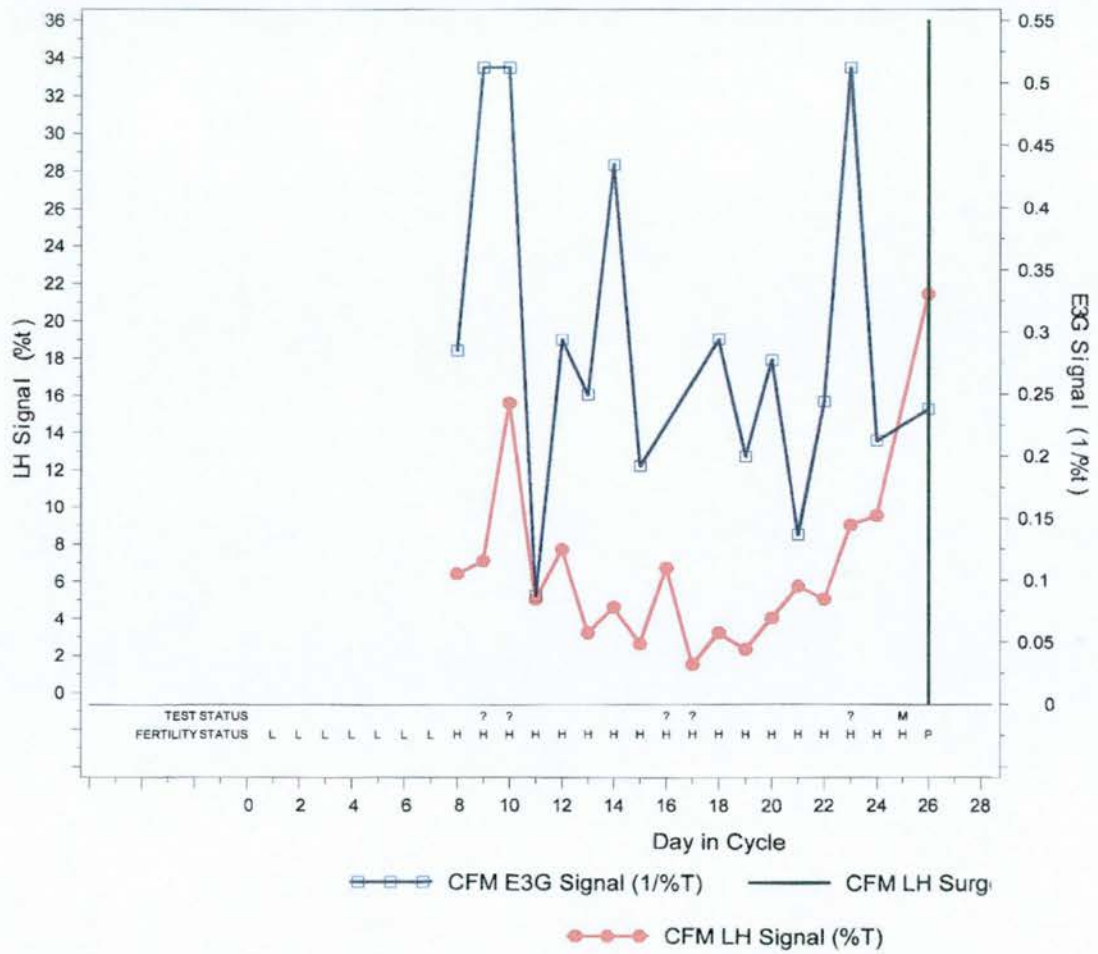
Thirty two volunteers were treated with a single dose of 200 mg of mifepristone administered in luteal phase of the cycle as their sole method of contraception between one and seven cycles. They contributed a total of 178 cycles, and in 167 cycles mifepristone was administered. Eight women withdrew from the study before completion, two women moved out of the area, three ended their relationship, two conceived during the study and one lost confidence in the method.

Two clinical pregnancies occurred in the 178 cycles studied. The first pregnancy was a true treatment failure, which occurred in a woman (para 1) who took mifepristone on day 14 (LH +2) of her first treatment cycle. She opted for a surgical termination of pregnancy, which was performed at eight weeks of gestation (confirmed by ultrasound scanning). In the second woman (para 3), an LH surge was not identified in her third study cycle hence she did not receive treatment with mifepristone (Figure 3.4), menses did not occur and on day 37 after her last menstrual period an ectopic pregnancy was diagnosed and treated surgically. In a third woman a biochemical pregnancy was diagnosed (serum β hCG only rising to 34 U/L), which was spontaneously and completely aborted by day 34 of the third study cycle after taking mifepristone on day 14 (LH +2). This woman continued in the study and completed 6 treatment cycles.

Figure 3.4 *This figure illustrates the down loaded test data from the CPEFM used by the woman who missed the LH peak due to monitor error*

The E3G and a subsequent low LH peak occur "too early" in the cycle hence the monitor does not declare peak fertility. The closed circles represent the LH signal, and the open squares represent the E3G signal. Fertility status is indicated as

L = low fertility, H = high fertility, & P = peak fertility. The solid green line indicates the monitor declaring an LH peak because of cross reactivity of rising βhCG with the LH assay.



The mean frequency of sexual intercourse was 1.8 episodes per week in 167 treatment cycles in which sexual activity was recorded. If we assume the probability of pregnancy in the treatment group is similar to the control group (0.25 - 0.32), the expected number of clinical pregnancies during the 178 cycles (in which 140 were exposure cycles)

studied should be between 35 – 48.3. The observed number was 2. Therefore, the efficacy of the method is 94.3% (95% confidence interval 75.4 – 98.7) - 95.9% (95% CI 82.5 - 99.0). When calculating the efficacy of the method, we excluded the 29 cycles during women were not exposed to a risk of pregnancy, and the three cycles in which mifepristone was taken in the follicular phase (Table 3.2).

3.3.3 Contraceptive efficacy of luteal phase administration of mifepristone

In 145 cycles in which mifepristone was taken in the early luteal phase (within 2 days of the urinary LH surge) 117 were exposure cycles. Exposure status was unknown in eight cycles and in 20 cycles women were not at risk of pregnancy. In the 117 exposure cycles, there was only one clinical pregnancy.

In 19 (10.7%) cycles, the monitor declared no LH surge but mifepristone was given as coitus had taken place during the fertile period of the cycle (calculated according to the usual cycle length and usual day of LH surge). Occurrence of ovulation was confirmed by serum progesterone of >5 nmol/L in all 19 cycles and treatment was administered prior to day 21 of the cycle in each case (between day 13 – 21 of the cycle (Mean 16.9 (SD +/-2.1) days). There were no pregnancies in these cycles.

If the probability of pregnancy in all exposure cycles in the study is 0.25 - 0.32 (the same as that of the control group), 34 - 46 clinical pregnancies would be expected in the 136 ovulatory cycles in which mifepristone was taken in the luteal phase. The observed number was one. Hence, the contraceptive efficacy of luteal phase mifepristone is between 97.1% (95% CI 78.00 – 99.6) and 97.8% (95% CI 83.9 – 99.7) (Table 3.2).

3.3.4 Performance of the home use hormone monitor

In 140 treatment cycles an LH surge was identified by the monitor, which equates to 90.9% LH surge detection when calculated for perfect use cycles; and 80.5% when

Table 3.2 Treatment cycle details.

	Total number of cycles	Exposure cycles	Unknown exposure	No Exposure
Mifepristone administered	167	136	8	23
• In follicular phase	3	0	0	3
• In luteal phase	164	136	8	20
Early luteal phase	145	117	8	20
LH +2	127	100	7	20
LH +1	17	16	1	0
LH+0	1	1	0	0
In luteal phase (Unknown LH status)	19	19	0	0
• Mifepristone not given	11	5 (LH surge missed, at risk of pregnancy but after day 21)	0	6 (Anovulatory cycle n = 1 LH surge missed & no risk of pregnancy n = 5)
Total	178	141	8	29

imperfect use cycles are also included in the total. In 127 cycles this was confirmed by a subsequent rise in serum progesterone of >5 nmol/L in the early luteal phase. This information was not available from 9 cycles (blood samples lost or not collected). In the remaining 4 cycles serum progesterone was between 2 – 5 nmol/L, one or two days following the urinary LH surge as detected by the monitor. This may have been due to an early detection of the first significant rise in urinary LH. None of these five cycles were prolonged after taking mifepristone hence; it is unlikely that they were anovulatory.

There was a total of 38 (21.3%) cycles in which an LH surge was not detected. Among them, one (0.6%) was an anovulatory cycle, defined by serum progesterone not rising above 5 nmol/L in the mid-luteal phase. In three (1.7%) other cycles we administered mifepristone on day 19, before the monitor had identified an LH surge. Serum levels of progesterone (taken on the day of administering mifepristone) confirmed that in these cycles mifepristone was administered in the follicular phase. All three cycles were prolonged (43 - 52 days).

In the remaining 34 cycles an LH surge probably occurred (as suggested by a rise in serum progesterone of >5 nmol/L) but was not identified by the monitor. Fourteen were missed due to monitor method failure (7.9%) and 20 were a consequence of imperfect use of the system (11.2%).

3.3.5 Cycle length

Mifepristone, when given in early luteal phase, did not significantly affect the cycle length ($p = 0.35$). The mean of the usual cycle length was 28.3 days (SD +/-1.3) and during the treatment cycles it was 28.0 days (SD +/-1.9).

3.3.6 Side effects

Women kept a record of vaginal bleeding in 139 out of the total 144 cycles where mifepristone was taken on LH +2. Mifepristone induced vaginal bleeding within 72

hours in 21 cycles (15%). In further 19 cycles, volunteers took mifepristone in the luteal phase but the LH status was not known. In 17 of those cycles (>89%), mifepristone induced a vaginal bleed.

Serum progesterone values in blood samples taken just prior to mifepristone administration were available for 136 cycles. The mean serum progesterone value was significantly ($p < 0.0001$) higher in those cycles where mifepristone induced bleeding when compared to the mean value for the cycles without bleeding (21.72 (SD +/-9.04) nmol/L Vs. 13.33 (SD +/-6.23) nmol/L).

Two women spontaneously reported improvement of their pre-menstrual symptoms during cycles in which mifepristone was administered, while one reported worsening. In one woman hepatic alanine aminotransferase (ALT) was elevated at 103 U/L (normal range 10 – 40 U/L) at the end of the study but returned to normal within 2 months. One woman complained of diarrhoea 12 hours post mifepristone in one cycle, 3 reported menstrual cramping within 72 hours of taking mifepristone; 2 women reported a reduction in menstrual blood loss.

3.4 Discussion

A single dose of 200 mg of mifepristone administered once a month is an effective contraceptive method with an overall efficacy of 95% increasing to 97% if administered at the correct time (i.e., the early luteal phase). Thus, our results are in agreement with the findings of the previous study by Gemzell-Danielsson (Gemzell-Danielsson *et al.* 1993).

One criticism of previous work in this field has been the lack of a suitable control group for the subjects studied. Unlike the Gemzell-Danielsson study, we were able to compare the results with a contemporaneous control group using the same methodology in the same cultural setting. In this control group, if sexual intercourse took place on a fertile

day the probability of a pregnancy was 0.25 - 0.32. The calculated probability of pregnancy in a cohort of couples monitored during a study of natural family planning (WHO 1983) was 0.486 if intercourse took place 3 days prior to and a day after the peak day of mucus discharge. The difference in the probability of pregnancy between our study and a variety of other published series (Table 3.3) may be explained by the fact that we have extended our definition of the fertile period to 6 days (3 days prior to the urinary LH surge until 2 days after). Other authors (Wilcox *et al.* 1995) have shown that the likelihood of conceiving during an ovulatory cycle to be 0.37 (95% confidence interval 0.31 to 0.48) if daily sexual intercourse took place during a 6 day fertile period (four days before and a day after ovulation). The lower frequency of intercourse in our group (untimed intercourse averaging 1.7 per week) may also explain the lower probability of pregnancy.

Table 3.3 Probability of clinical pregnancy

	Number of exposure cycles	***Number of pregnancies***	***Probability of pregnancy***
Wilcox study+	129	34	0.26
Our control group+	37 - 48	12	0.25 – 0.32
Our treatment group+ (monitor + mifepristone)	140 - 151	2	0.01
Our treatment group+ (mifepristone in luteal phase)	136 - 143	1	0.007
G-D study group*	124	1	0.008
WHO study*	72	35	0.48

*The length of the fertile period defined as 4 days

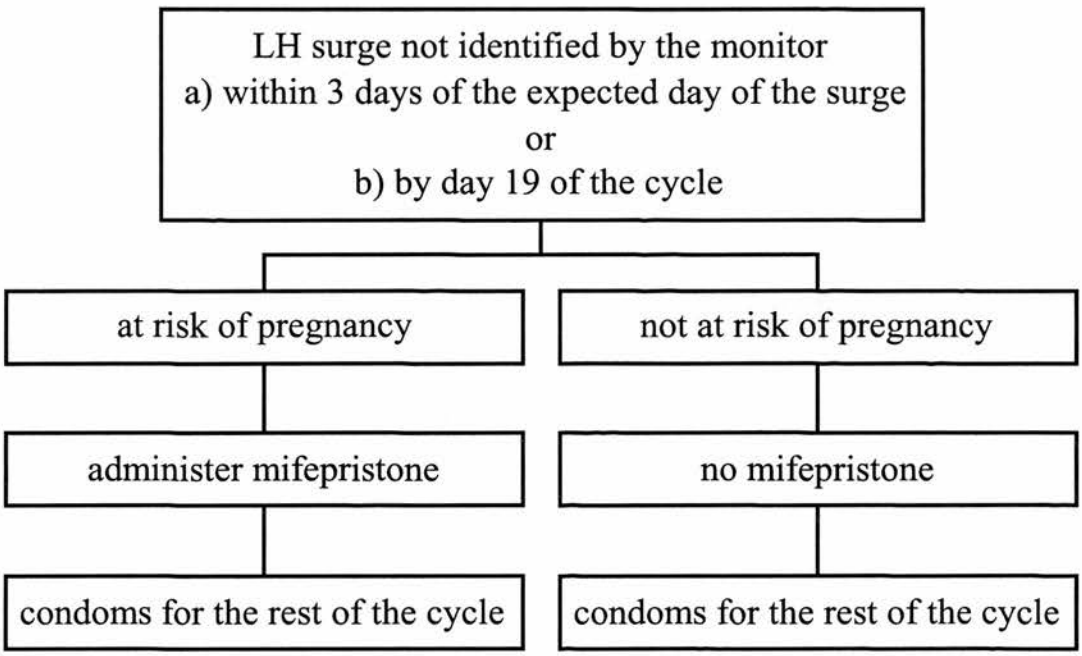
+The length of the fertile period defined as 6 days

The limiting factor in this once-a-month approach to administering anti-progesterone is the accurate detection of the LH surge. Clearly, the failure to detect accurately the LH surge has a big impact on the overall effectiveness of the method. Using laboratory assay of LH in blood or urine to identify ovulation is neither practical nor convenient for long term use in the general population. The monitor provided us with an opportunity to overcome these problems. Gemzell-Danielsson reported 49% accuracy using home LH detection sticks. Although the monitor performed better (over 80.5% accuracy), both of these methods remain below the required standard. We studied 32 women over a total of 178 cycles. Imperfect use of the system accounted for failure to identify an LH surge in 11.8% cycles while 7.9% were due to monitor method failure. Compliance difficulties are associated with all contraceptives and non-compliance in approximately 12% of cycles is probably no worse than with any other method which demands action from the user, for example, compliance rates reported from oral contraceptive pill users range from 3.4% - 100% (Wheble *et al.* 1981, Hamilton *et al.* 1989, Molloy *et al.* 1985). Although our study population consisted of women who were motivated and committed and some of them already had experience in using natural family planning methods, they found the short, inflexible testing window set on day 1 of the cycle to be particularly demanding. This is inconsistent to couples using the monitor in order to get pregnant (Bonnar *et al.* 1999). The prevalence of imperfect use is likely to rise in the general population compared with that typical of a research study.

During the course of the study we developed an algorithm (Figure 3.1) for the administration mifepristone if an LH surge was not identified. In 19 exposure cycles (out of 28 cycles in which an LH surge was not identified) mifepristone was administered using this algorithm and there were no pregnancies. Given that the methods available to be used in real life to time the administration of mifepristone cannot be 100% accurate, such an algorithm will be essential to deal with a missed LH surge.

In our study, mistimed administration of mifepristone led to predictable effects. When administered during the proliferative phase of the menstrual cycle, mifepristone inhibited follicular development, and delayed the mid cycle LH surge, leading to a delay in ovulation and subsequent prolongation of the menstrual cycle (Liu *et al.* 1987, Luukkainen *et al.* 1988, Swahn *et al.* 1988). Ovulation may occur later in that cycle, leaving women at risk of conception. In our study, when administered in the late follicular phase (in error) in 3 cycles, mifepristone prolonged the cycle length (43 – 52 days). The women were advised to use condoms for the remainder of that cycle and none of the three cycles resulted in pregnancy.

Figure 1. Algorithm for administering mifepristone when the LH surge is not identified.



Administration of mifepristone in the mid or late luteal phase induces a bleed within a few days of treatment, which may or may not be followed by a second bleed at the time of expected menstruation (Shoupe *et al.* 1987; Swahn *et al.* 1988). The disturbance in vaginal bleeding induced by a method of contraception is an important side-effect because of its potential impact on acceptability. In our study, in 17 out of the 19 cycles

where mifepristone was taken after ovulation (the LH status unavailable and probably later than on LH +2), inter-menstrual vaginal bleeding occurred (89.5%). Moreover, there was an increased risk of bleeding seen in those women who may have taken mifepristone slightly later in the LH +2 window. The mean serum progesterone concentration was significantly higher in those women who had bleeding after taking mifepristone within LH +2, when compared with those who did not. The higher serum progesterone value in some on LH +2, could be due to a delayed identification of the first significant rise in urinary LH, or because of a more rapid increase in serum progesterone due to early ovulation. Nevertheless, in our group of women, in all cycles where mifepristone induced a vaginal bleed, a second bleed occurred at the time of the expected menses. Therefore, while the bleeding may have been inconvenient, it did not jeopardise efficacy or continued use of the method. There was less inter-menstrual bleeding (15% of the cycles) reported in our study when mifepristone was taken within LH +2, less than half of that reported by Gemzell-Danielsson *et al.* (32%). This is possibly due to the fact that the majority of women in our study received mifepristone at the correct time. In their study, in 51% of the cycles, mifepristone was taken between 3 and 5 days after the LH surge. Anyhow, this predictability in the bleeding pattern following luteal phase administration is likely to be mere inconvenience, as opposed to the follicular phase administration.

The mean serum progesterone value was significantly more in those women who had mifepristone induced bleeding in the early luteal phase, when compared with those who did not. The higher serum progesterone value in some on LH +2, could be because of delayed identification of the first significant rise in urinary LH, or because of a faster increase in serum progesterone due to early ovulation.

The upset in menstrual pattern that may follow administration of mifepristone in the proliferative phase or in mid / late luteal phase can be unacceptable to women. However, there is new evidence (Mations *et al.* 2002; Brown, A. unpublished observation) that the

time window for administering once a-month mid-cycle mifepristone is wider than previously thought. This may be tested in the future where mifepristone is taken on a specific day of the cycle, arbitrarily chosen according to each woman's usual menstrual cycle length.

Women are not exposed to the risk of contraceptive failure unless they have intercourse on a fertile day. Therefore, when calculating the contraceptive effectiveness of the method we excluded those cycles where the women reported that no intercourse had occurred during the fertile period and those cycles where insufficient information was available to determine whether intercourse has taken place. In our study, women marked only the days in which they had intercourse on the diary card, hence leaving no way to distinguish the days they forgot to mark sexual activity from those days when they actually did not have any. One might object that those who did not keep a record, still may have had intercourse during the fertile period and were nevertheless at risk of pregnancy. On the other hand, complex and more detailed diary cards may encourage errors of omission.

In conclusion, the use of the combination of home use fertility monitor with once a month administration of mifepristone (especially if mifepristone is administered at the early luteal phase) is an attractive contraceptive option with minimal side effects. However, to be an effective contraceptive method, the women have to be committed to using a device, which identifies the LH surge, in order that the pill can be taken at the correct time in the cycle. Whilst this regimen may be acceptable to motivated women, it may be regarded as too complicated for others to adopt on a routine basis. There was evidence of such non-compliance in this study, with 11.2% of LH surges being missed as a consequence of imperfect use of the monitor. Unfortunately, it is difficult to envisage how an easier way of defining the correct timing, which obligated less compliance, could be devised.

CHAPTER 4

SEX, LIES AND NON-COMPLIANCE

"Keep watch also on the faults of the patients, which often makes them lie about the taking of things prescribed"

Hippocrates.

4.1 Introduction

When the first woman on earth chose to contradict the instructions of her Provider in the Garden of Eden, expecting her descendants to comply perfectly with a contraceptive regimen may seem rather unrealistic. However, regardless the culture, race or the religious beliefs of a woman, unintended pregnancy is undoubtedly a major life-changing event. The consequences of such pregnancy generally make it unwelcome. Non-compliance with a particular contraceptive method is linked with an increased risk of unintended pregnancy (Rosenburgh *et al.* 1995). Therefore, conventionally it has been assumed that the users of contraception are 'highly motivated' because the consequence of non-compliance – pregnancy - is so obvious, and so significant. Studies involving organ transplant recipients have shown however, that no consequence of poor compliance is severe enough - not even the rejection of a transplanted kidney - for all patients to follow their prescribed regimen reliably (Rovelli *et al.* 1989). Converging evidence from all disciplines, in the treatment of chronic disease that requires suffers to adhere to particular medical regimens over a long period of time, indicates that poor compliance is pervasive (Haynes 1979). Furthermore, women on contraceptives are not suffering from a disease.

The studies that have evaluated the patient's compliance with oral contraceptives show a wide range between the highest (100%) and the lowest (3.4%) compliance rates (Wheble *et al.* 1981, Molloy *et al.* 1985; Hamilton *et al.* 1989). This discrepancy may be due to the methodology of the studies as well as the differences in the patient characteristics - there is no "gold standard' measurement for patient compliance (Spilker 1991).

Poor compliance – in both clinical practice and research – is associated with a number of factors. Though several patient characteristics (e.g. age, education, socio-economic background (Rapoff 1986; Meichenbaum & Turk 1987; Potter *et al.* 1991), characteristics of the treatment regimen (e.g. frequency of dosing, side effects (Puller 1988; Rapoff *et al.* 1989; Cromer *et al.* 1989)) and outcome characteristics (e.g. treatment of incurable or terminal illnesses (Spilker 1991)) have been associated with

non-adherent behaviour, there are no reliable and universally applicable predictors of non-compliance (Peck & King 1982; Luscher *et al.* 1985; Haynes 1986). Given the relatively crude measures of compliance that are available, caution should be taken when trying to correlate compliance with the outcome.

When a contraceptive method is used imperfectly, its effectiveness may differ from that during perfect use (Dominik *et al.* 1999). Clinical trials to determine the efficacy of new contraceptive regimens depend on full co-operation of the volunteers. Little is known about the prevalence of non-compliance in contraceptive trials. In these trials, volunteers are often required to keep daily records of events such as pill taking, sexual activity, vaginal bleeding and side effects in order to comply with a protocol. Contraceptive efficacy is usually compared with the estimated conception rates among non-users, which themselves directly rely on the accuracy of menstrual cycle data. Few women in real life regularly keep records of their menses or sexual activity hence, reporting errors are common. Moreover, incorrect reports of perfect compliance can under estimate use-effectiveness rates. Investigators generally depend on self-reporting (SR) of non-adherence behaviour to detect the prevalence of imperfect use.

We observed the behaviour in 32 women who took part in a trial assessing the efficacy of a novel method of contraception (Hapangama *et al.* 2001b). The women took 200 mg of mifepristone two days following the mid-cycle LH surge (measured in urine) as a once-a-month contraceptive pill. Mis-timed administration of mifepristone can disrupt the menstrual cycle, and can also leave the women at risk of conception. A home use fertility monitor that could store data on daily testing events was used to time the administration of mifepristone. While making minimal demands on the users, the method provided objective, long-term data on their routines. Since the investigator saw participants once each cycle throughout the study, we were able to compare the results of this method with the traditional self-reported incidence of compliance.

4.2 Subjects & Methods

4.2.1 Subjects

Data were collected during a study assessing the feasibility of administering mifepristone as a once a month contraceptive pill and detailed study methodology is reported elsewhere (Chapter 3; Hapangama *et al.* 2001b). Thirty-two sexually active women were enrolled from a large family planning clinic in Edinburgh. All subjects gave written informed consent to participation and the Lothian research ethics committee approved the study. Data was collected from a total of 178 cycles with each subject contributing between one and eight cycles. It was not possible to retrieve compliance data from the monitors in 28 of the study cycles, due to infrequent downloading of the monitor, and lost or broken devices. The study started on day 1 of the menstrual period following screening, and lasted for up to seven consecutive menstrual cycles in which subjects took a single tablet of 200 mg mifepristone once per month. The timing of mifepristone depended on detection of the LH surge, which in turn depended on compliance with daily urine testing.

4.2.2 Methods

All women underwent screening at the time of recruitment including routine physical and gynaecological examination. They were provided with a home use fertility monitor (Unipath, Bedford, UK). The system comprises a hand-held monitor and disposable dual-assay urine test sticks, and is used to detect simultaneously LH and E3G levels in early morning urine. The system will delineate three levels of fertility (Low, High and Peak Fertility) according to the optical signal changes detected. At the start of each menses, the subjects pressed the 'm' button on their monitor to initiate that cycle of use, at a time suitable for testing the first urine of the day. For the rest of the month, the subjects were required to consult the monitor display each morning (three hours either side of the time when 'm' button was set) to determine whether they needed to perform a test that day. Outwith this six hour time window the system would not accept a test. The monitor requests one test every day for up to a total of 10 or 20 tests, depending on the length of the woman's cycle, and the timing of her LH surge. Embedded software within

the monitor collects, analyses and, stores data for several months (Figure 2.1; Figures 3.2 & 3.3).

The correct way to use the monitor (including the importance of the testing window) was demonstrated to all subjects at the time of recruitment and written information was given. Subjects were advised to contact the investigator immediately if they were not able to perform a test during a critical period. The investigator was available by telephone seven days a week.

Subjects were reviewed by the investigator each cycle, on day LH +2 when mifepristone was taken.

All subjects kept a menstrual record card, recording all vaginal bleeding experienced during the study and the days on which they had sexual intercourse. They also marked the day of the LH surge as identified by the device and the day of taking the study medication.

The following definitions were created for the purpose of the study.

The period of time between the calculated earliest day an LH peak is likely to occur and the day of the actual LH surge in each cycle was defined as the **Critical period** for each patient. **The Fertile period** of the cycle was defined as three days before until two days after the urinary LH surge (LH -3 to LH +2). **Exposure cycles** were cycles in which women reported having sexual intercourse at least once during the fertile period.

4.2.3 Statistical Methods

Some of the compliance data were summarised for descriptive purposes using numbers of cycles or tests as denominators. However, statistical inference was carried out on data aggregated to patient level, in order to take account of possible heterogeneity in behaviour amongst patients, which might have invalidated analyses using data from individual cycles or tests. Thus percentages of tests missed were calculated for each

patient, and these were tested for association with demographic data using Pearson correlation. Similarly, percentages of tests missed at different stages of the cycle, and when on and off the treatment regimen, were compared by paired t-tests. A two–sample t-test was also used to compare the percentage of tests missed in patients who dropped out and those who did not.

4.3 Results

Table 4.1 shows the demographic characteristics of the women who took part in the study.

Table 4.1 *Demographic data*

Demographic feature		***N = 32***
Age (years)	Range	18 – 39
	Mean (+/- SD)	30 (+/-5.4)
BMI	Range	19 – 38
	Mean (+/- SD)	23.6 (+/-4.3)
Smokers	(%)	7 (21.9)
Non-smokers	(%)	21 (65.6)
Ex-smokers	(%)	4 (12.5)
Previous pregnancies	1+ (%)	19 (59.4)
	Never pregnant (%)	13 (40.6)
Ever abortion (%)		15 (46.9)
Married / Co-habiting (%)		28 (87.5)
Single (with a regular boy friend) (%)		4 (12.5)

162 cycles were analysed, out of which 150 were study cycles. Data were collected during an additional 12 cycles in which subjects were using a barrier method throughout the cycle and mifepristone was not given.

In total 2013 tests were requested by the monitor during the 162 cycles analysed (12.4 tests per cycle, 95% C.I. 11.8 - 13.0), and 494 were missed (24.2% (95% C.I. 16.5 - 31.5). On average three tests (95% C.I. 2.4 - 3.6) per cycle were missed.

4.3.1 Compliance during the intention to treat cycles

During the 150 cycles, in which women were relying on the study method as their only contraception, a total of 1816 tests was requested by the monitor and 411 tests were missed (22.6% (95% C.I. 15.2 - 30.1).

Compliance before and after identifying the peak

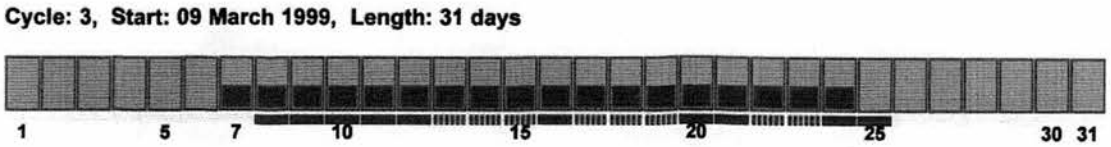
In the days before the women knew an LH surge had occurred, 23% of the requested tests were missed (95% C.I. 16 - 30)) (260 out of a total 1160). Women were not more likely to miss tests before the surge than after it ($t = 0.57$, NS).

Concordance of monitor data with the self-reported data

In 68 cycles (42%) women failed to test at all on a day of the cycle, which was critical to the accurate detection of an LH surge. In 27 of these cycles the monitor did not detect an LH peak. In the remaining 41 cycles despite critical tests being missed the monitor did detect an LH peak. Women admitted to not performing tests in 24 cycles (14.8 %). In the other 44 cycles women did not report missed tests and only admitted to it once the investigator showed the downloaded monitor data to them. Some women actively fabricated the information, which they reported to the investigator. In one of these cycles when contacted, a woman declared detecting an LH peak on a day (day 13 of the cycle) when she had not performed a test at all (Figure 4.1). Another woman performed the tests correctly and identified an LH peak on day 13 but forgot to inform the investigator and failed to obtain the mifepristone tablet as per protocol, when contacted on day 19 of the same cycle, she claimed that the monitor had not detected an LH peak.

Figure 4.1 This figure illustrates the monitor display as seen by the volunteer each day

Empty box = low fertility, Half filled box = high fertility.
Underneath each box, a solid line represents a correctly performed test while a missed / inaccurately performed test is represented by a broken line. Volunteer using this particular monitor neither performed a test, nor did the monitor show peak fertility on cycle day 13. However, she declared detecting peak fertility on day 13 (see Figure 3.2 for perfect compliance).



Compliance during the critical days

Compliance with urine testing as monitored by the system was significantly higher during the critical period (15.6% (95% C.I. 9.5 - 21.7) when compared to the non-critical days (27.5% (95% C.I. 17.9 - 37) (paired t-test, t = 3.64, p <0.01). The self reported percentage of missed tests during the critical period was 2.7% (95% C.I. 1.0 - 4.3) which was significantly lower than that detected by the monitor (t = 4.48, p <0.001).

4.3.2 Compliance during the study suspension interval

In six out of these 12 cycles, the monitor was not able to identify an LH peak due to imperfect use. During this period, a significantly high percentage of tests (41.2 % (95% C.I. 22.4 – 60.0)) were missed when compared to the study cycles (22.6% (95% C.I. 15.2 - 30.1) (t = 2.9, p = 0.015).

4.3.3 Compliance and sexual activity

There was no correlation between the frequency of sexual intercourse per cycle and the number of missed tests per cycle (correlation coefficient was +0.01). The percentage of missed tests were compared between the exposure and non-exposure cycles in 12 women who had at least one exposed and one unexposed cycle. There was no significant difference (paired t-test, t = 0.06, NS, 95% C.I. for differences between exposed and unexposed –8.3 to +7.8) in compliance during exposed and non-exposed cycles.

4.3.4 Demographic features and compliance

Age was negatively correlated with the percentage of missed tests – younger women missed more tests ($r = -0.36$, $p < 0.05$). There was no apparent relationship between compliance with the method and the number of pregnancies, number of previous abortions, or number of living children (Table 4.2). All women included in the study had some form of tertiary education and were either employed or were undergoing tertiary education during the study period.

Table 4.2 *Demographic data and compliance*

Demographic feature		***Number***	***Mean % non-compliance***
Age	18 – 24	5	36.8
	25 –29	11	24.2
	30 – 34	7	16.8
	35 - 39	8	15.2
BMI	19 –20	6	13.7
	21-25	16	27.9
	26-38	7	14.3
Smoking	Current	7	24.3
	Never	19	21.7
	Ex	4	18.4
Previous pregnancies	1+	17	22.6
	Never	14	21.9
Ever abortion	Yes	14	25.3
	No	17	21.9
Married/ Co-habiting		27	22.4
Single		3	18.1

4.3.5 Compliance among dropouts

We analysed cycle data from the seven women who discontinued the study before completion. They missed a significantly higher percentage of tests (44.4%) when compared with the other 25 women who completed the study (17.4%) (95% C.I. of the differences between the two percentages 10.6 – 43.3) ($t = 3.39$, $p = 0.002$). The dropouts missed tests on a critical day in 16 out of the 22 cycles although admitted to missing tests in only four. Therefore, the self reported incidence of non-compliance during the critical period was 18.1%, while the monitor detected incidence was 72.7%.

4.4 Discussion

The home use fertility monitor offered us an important methodological advance in providing reliable data on the incidence and the magnitude of non-compliance with the contraceptive method studied, and information on when exactly the imperfect use occurred. We sought to achieve significantly high compliance rates with employing this monitoring system in a contraceptive regimen, which appeared to be acceptable to women (Rimmer *et al.* 1993; Glasier *et al.* 1999). Women were counselled at the start of the study regarding the importance of performing urine tests accurately in order to identify the time to administer mifepristone. They were aware that if mifepristone was not taken, they would be at risk of pregnancy. Since the teratogenic effects of mifepristone is not known women considering participation in the study were advised that if pregnancy occurred they should consider termination. Despite this, the information collected from the monitor demonstrates that women failed to perform 24.2% of the tests in the 162 cycles analysed. Moreover, in 42% of the cycles women missed tests during a day that an LH surge was likely to have occurred, i.e. at an absolutely vital time for contraceptive efficacy.

Although non-compliance is common, by inviting only a selected group of women (all with tertiary education) who appeared to be motivated and committed we sought to exclude those who might be poor compliers. We expected the positive health benefit, i.e. avoidance of pregnancy from our 'new' treatment may become their motivation to

comply with the protocol. Interestingly, the medical profession is reportedly poor in identifying non-compliers (Gilbert *et al.* 1980). Users of our method were reasonably young (mean age 30 years) and were healthy. The serious outcome of non-compliance (pregnancy) was rare. Women may have found daily requirement for urine testing using the monitor on a long-term basis to be complicated or inconvenient. Side effects of the method (although fewer than in most other methods available) may not have been acceptable to them or they may not have had faith in the effectiveness of the method although this seems unlikely since they did not revert to condoms. Considering these odds, it is not difficult to infer why compliance problems were frequent.

For a volunteer, taking part in a research study usually involves extra effort. Women in our study were required to attend clinics, answer questions honestly, test samples accurately as requested (daily urine), allow required tests to be performed (serum progesterone assay), obtain medicines prescribed and keep accurate records of events (sexual intercourse, menstrual bleeding etc) in order to comply with the study protocol. Common sense suggests that with increasing experience the women may trivialise the need for testing on days in which an LH surge was unlikely to have occurred. All women were made aware of the critical period for testing urine at the start of the study and as expected, women missed fewer tests during this period. Incomplete understanding of how to be compliant with a regimen has been suggested as a common reason for poor compliance (Rosenberg *et al.* 1995). Women in our study were given detailed verbal and written information on using the monitor at the start of the study. The importance of accurate use of the device according to manufactures guidelines was stressed at each monthly visit. Since there was no significant difference in the percentage of missed tests between the first study cycle and the last study cycle, it is difficult to support the suggestion that in the earlier cycles women were less confident about how to use the method or more confident in their ability to guess the correct time for testing (Lusher *et al.* 1985).

There was a highly significant difference between the patient self-reported percentage of missed tests (2.6 %) and that detected by the monitor (15.6 %) during the critical period (Figure 4.2 & 4.3). This discrepancy was even greater among the seven women who discontinued the study before completion, and is consistent with previous studies that reported significant overestimation of compliant behaviour with SR (Potter *et al.* 1996). In 1996, Potter and colleagues studied SR incidence of missed pills with the data from an electronic device with diary entries among a group of OC users, and the compliance ranged from 25% (Electronic Device data) to 66% SR. Patients tend to tell the doctors what they think the doctor wishes to hear. If they assume that doctors perceive patient non-adherence as a judgmental disappointment, they may feel guilty to report non-compliance.

Although, older women tended to miss fewer tests, the other demographic characteristics such as parity, previous abortions, number of living children and educational background did not correlate with the number of missed tests. Others have also reported a positive correlation of increasing age with adherence behaviour (Waterhouse *et al.* 1993). Neither did frequency of sexual activity or exposure to the risk of pregnancy correlate with the missed tests. However, since the information about sexual activity was only collected by self-reporting, this association should be accepted with caution.

Most investigators work on the assumption that a patient who complies with one aspect of a clinical protocol (e.g. attending the clinic as directed) also adheres to all other aspects of the study, though this might not always be the case. The monitor supplied data on daily testing of urine, which was only one part of the contraceptive method. We cannot infer from those data, how well the women in our study complied with the rest of the protocol, such as recording daily events (sexual intercourse, vaginal bleeding). With no means of testing this, for validity of our results we have to depend on our volunteers being truthful. Therefore, in future we have no other option but to work towards forming a true therapeutic alliance with our volunteers; and to come to an agreement with our patients rather than to impose a prescription or a protocol upon them.

In conclusion, the use of microelectronics monitoring systems such as the home use fertility monitor may improve our understanding of the extent of the problem of patient non-compliance, providing precise objective information that no other monitoring technique can produce. This understanding will empower us as health care providers, to adopt a no fault approach to behaviour relating to non-compliance and establish "a tailored consensual regimen" with the user which they are able to adhere to (Fink, 1976). This provides the opportunity to make the optimum use of potentially effective treatments and legitimate research evidence. Perhaps a small price to pay for such a return!

Figure 4.2 Information collected from the monitor and the diary card from a 31-day cycle in which, the LH surge was identified. Mifepristone was administered on day 20 (LH +2)

Panel 1: *Information downloaded from the monitor*

Level of fertility displayed to the woman on each day of the cycle.

Low fertility High fertility Peak fertility

Data on testing events.

Test performed Test missed

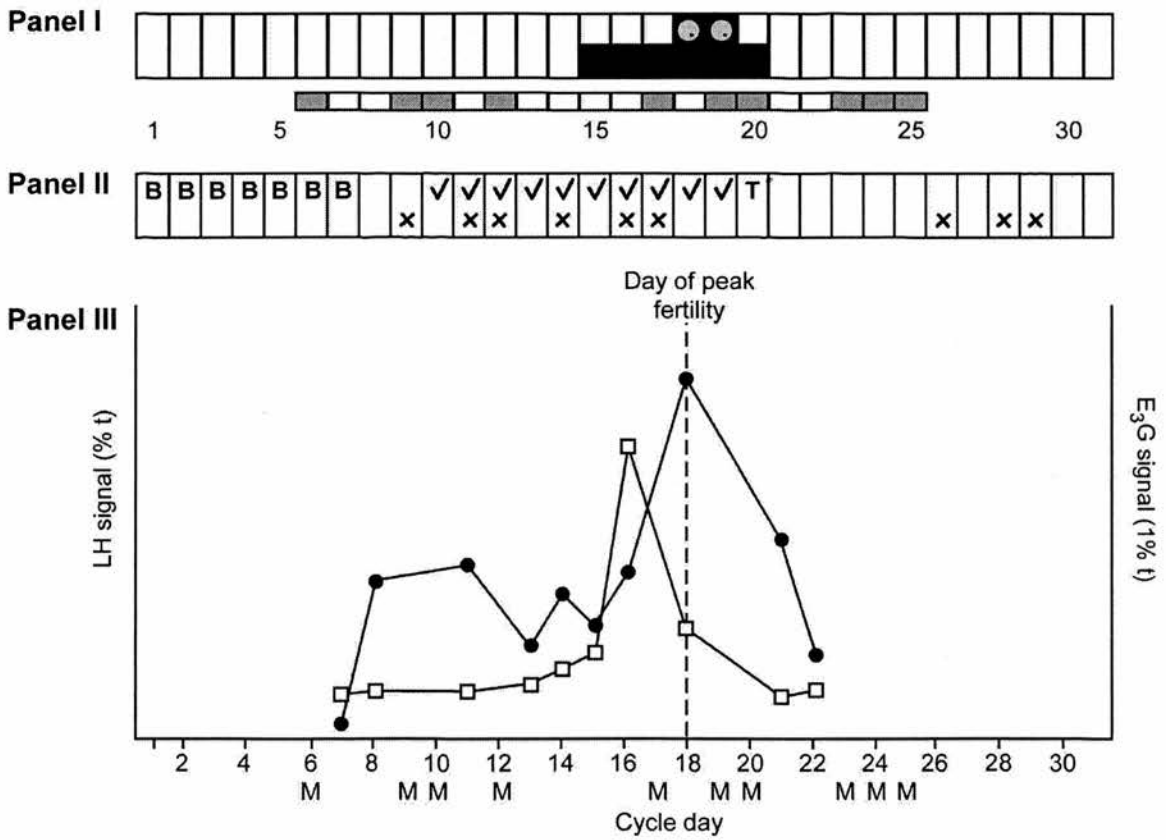
Panel II: *Data recorded on the diary card by the woman* ***(Self-report)***

B = days of vaginal bleeding X = days in which sexual intercourse had occurred

√ = tests done during the critical period T = day when mifepristone was taken

Panel III: *Downloaded information on signal levels of Luteinizing Hormone* ***(LH)*** *and Oestrone-3-glucuronide* ***(E3G)*** *levels.*

LH (●) E3G (□) M = Missed tests



**Note that on several days – the woman reported performing (√) the test when the monitor showed that she did not (M).

Figure 4.3 Information collected from the monitor and the diary card from a 35-day cycle. As a consequence of missed tests the monitor did not identify the LH surge and, mifepristone was not administered

Panel I: *Information downloaded from the monitor.*

Level of fertility displayed to the woman on each day of the cycle.

Low fertility High fertility

Data on testing events

Test performed Test missed

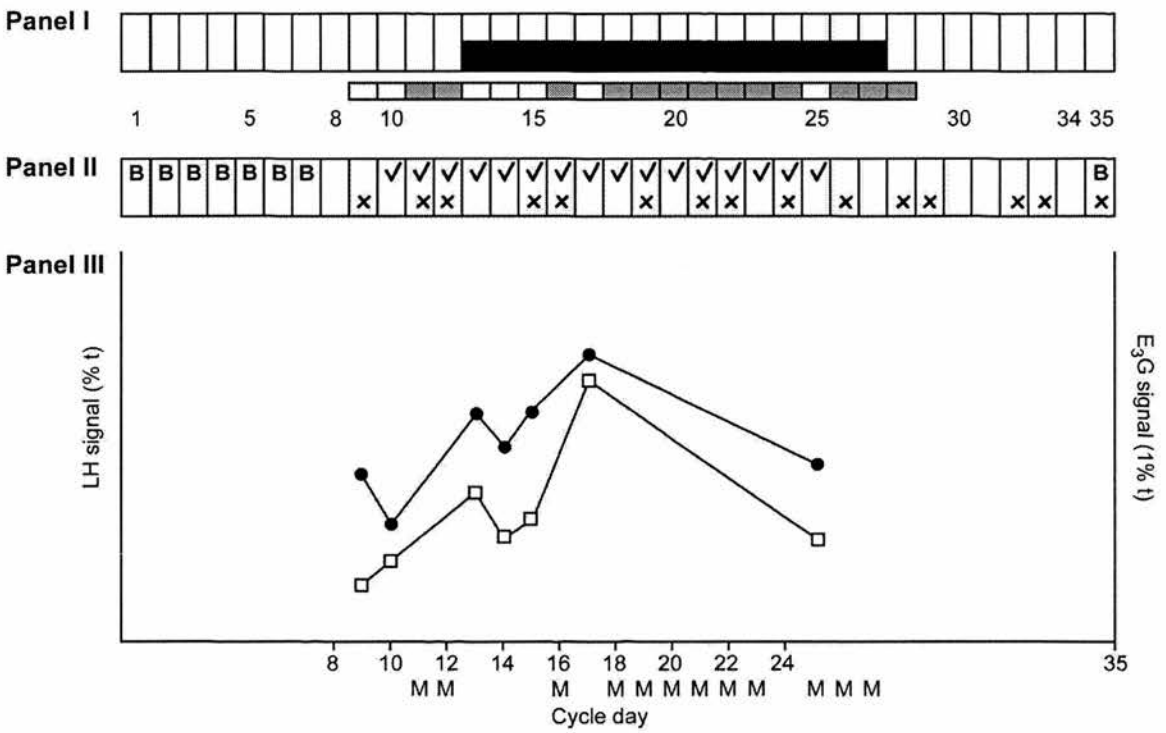
Panel II: *Data recorded on the diary card by the woman* ***(Self-report)***

B = days of vaginal bleeding √ tests done during the critical period

X = days in which sexual intercourse had occurred

Panel III: *Downloaded information on signal levels of Luteinizing Hormone* ***(LH)*** *and Oestrone-3-glucuronide* ***(E3G)*** *levels*

LH (●)E3G (□) M = missed tests



CHAPTER 5

THE ROLE OF A PERSONAL HORMONE-ASSAYING SYSTEM IN CLINICAL RESEARCH

5.1 Introduction

If sexual intercourse takes place on a fertile day of a woman's menstrual cycle, she may fall pregnant. The potentially fertile period in the cycle is determined by the time of ovulation. The duration of this fertile period however, is dependent upon the survival time of the sperm and the egg in the female genital tract.

The mid-cycle peak in the urinary levels of LH precedes ovulation by approximately 24 hours (Collins *et al.* 1996). A variety of self-detection kits or devices measuring urinary concentrations of LH has been used to determine the day of ovulation. The maximum survival time of the ovum is calculated to be less than 48 hours (Royston 1982). The rapid drop observed in the probability of conception after ovulation further supports a short life span for ova (Wilcox *et al.* 1995). The enlarging dominant follicle produces increasing levels of oestradiol in the late follicular phase (Kerin *et al.* 1981). Corresponding levels of E3G, the main metabolite of oestradiol - can be detected in the urine and have been used in conjunction with urinary LH to identify the potentially fertile period prior to follicular rupture and ovulation (Aldercreutz *et al.* 1982, WHO 1983, Schiphorst *et al.* 1985). Estimates using clinical data have suggested the number of fertile days in each cycle to be around 6 days, ending on the probable day of ovulation (Wilcox *et al.* 1995).

An EC can be effective by inhibiting or disrupting ovulation / fertilisation or interfering with the transport of the blastocyst, or inhibiting its implantation in the endometrium. Sex steroids have several effects on the ovulatory cycle and on the endometrium depending on the time of administration hence have been used as EC. Detailed understanding of the anti-fertility mechanism of these agents may help to select the most effective option for each individual depending on the time in the cycle when intercourse had occurred. Therefore, a reliable yet simple method of detecting the fertile period and the day of the ovulation is invaluable for research into EC.

The frequent and regular laboratory assays of steroid hormones in either blood or in urine have traditionally played a major role (in detecting the fertile period of the cycle, the cyclical hormonal changes, and the timing of ovulation) in research into female contraception. This approach is not only inconvenient for the volunteers but it is also expensive and time consuming to collect samples for daily assays. For example, usually the daily urine samples are collected and stored over several cycles and then analysed for hormone levels. Poor compliance with collecting specimens or wrongly labelled specimens may be difficult to detect, but if undetected can present difficulties in the interpretation of results. We analysed the practical aspects of using a microelectronic personal fertility monitor as an alternative to the laboratory based hormone assays, in a study that investigated the mechanism of action of an EC.

5.2 Subjects and methods

This was a prospective, randomised, double blind, crossover study undertaken in one centre. Twelve healthy women (mean age 33.3 (range 26 - 41 years)) with regular cycles (mean 27.6 days (range 25 – 30 days) and mean BMI 25.7 (range 20 - 34)) were recruited. They were all using a reliable non-hormonal method of contraception or were abstinent during the study. All subjects gave written informed consent to participation in the study, which was approved by the Lothian Research Ethics Committee.

A method was sought to provide a convenient means of identifying the fertile period prior to ovulation, and thereby time the administration of LNG. A home-use Fertility Monitor was used for this purpose. This product is similar to the Personal System of Contraception (Persona, Unipath Bedford UK) (Bonnar *et al.* 2000) in that it monitors E3G and LH in urine, but differs in that it has been specifically designed for maximising the chance of conception. Unipath Diagnostics Co., Princeton, NJ, markets the fertility monitor in the US and Unipath Ltd., Bedford, UK markets it in Britain as CPEFM (Figure 2.1).

This inter-active system comprises a hand-held monitor and disposable dual-assay urine test sticks, and is used to simultaneously detect LH and E3G levels in early morning urine. The monitor optically measures the intensity of the lines that form on the test sticks after sampling, and the system will delineate three levels of fertility (Low, High and Peak fertility) according to the optical signal changes detected. Low Fertility will be displayed from day 1 of the cycle, until the hormone levels rise above the baseline levels. A change from Low to High Fertility is triggered by detection of elevated E3G levels. The change from High to Peak Fertility is triggered by the detection of an LH surge. Peak fertility is displayed on the day of the LH surge and on the following day (Figure 3.2).

Each woman was studied during four cycles, and was issued with a monitor at the beginning of the study. Subjects were asked to use the CPEFM according to instructions, and familiarised themselves with the monitors by using it during a pre-study cycle to identify the days of high fertility and the day of the LH surge. They also recorded days of vaginal bleeding.

Data from the pre-study cycle was used to predict the timing of the LH surge and of the fertile phase during the study cycles and thereby to predict when treatment should be administered. The three study cycles followed immediately after the pre-study cycle. Six subjects were randomly assigned to treatment arm A and received LNG in the first study cycle and placebo in the third study cycle. The remaining six subjects were randomised to treatment arm B and received placebo in the first study cycle and LNG in the third study cycle. The second study cycle was a "washout" phase for all women during which time they also received placebo tablets (Figure 2.2).

The randomisation list was produced using SPSS Rv.Bernouilli function such that each study number was randomly assigned to either treatment arm A or treatment arm B with the same probability i.e. 0.5.

Each subject collected a sample of early morning urine daily from the first day of the first study cycle until and including the first day of the menstrual bleed signaling the end of the last study cycle. Samples were frozen and later assayed in batches (with all samples from one subject assayed in a single batch) for measurement of urinary LH, E3G and P3G.

Quantitative assessment of urinary LH was performed using a LH MAIAclone kit (BIOSTAT-DIAGNOSTICS, Stockport, Cheshire, UK). This method incorporates two high affinity monoclonal antibodies into an immunoradiometric assay system and offers a working range of 1.5 – 200 mIU/ml. Urinary P3G was measured using a direct enzyme immunoassay (working range 0.16 μg/L – 20 μg/L), while a direct immunoassay was used to measure E3G levels (working range 8.36 nmol/L - 2140 nmol/L. Intra-assay and inter-assay coefficients of variation were 6% and 10% for E3G, 10% and 12% for pregnanediol and 3% and 5% for LH respectively (Yong *et al.* 1992). Only the intra assay variability was relevant since the specimens for a given woman were analysed on the same assay. Geometric means of daily replicates were divided by the respective daily creatinine concentration to correct for variations in the dilution of the urine specimen.

During study cycles 1 and 2 women were asked to take the study medication on the first day of high fertility as identified by the monitor. However, by the third study cycle, the variation in the number of High Fertile days (range 0 - 9 days) meant that the monitor could not be used to administer medication in the fertile period in every cycle. Therefore, we had to adopt a different method of calculating the anticipated day of the LH peak for each cycle based on the monitor information from the previous cycles (including the pre-study cycle). Hence, in the third study cycle, the medication was taken two days prior to the anticipated day of the LH peak. In all cycles the first tablet was taken at 11.00 hours and the second at 23.00 hours. A sample of venous blood was collected 5 – 7 days after treatment, stored and later assayed for progesterone using Coat-A-Cont solid-phase

radio-immunoassay. The subjects kept a daily record of all vaginal bleeding experienced during the 4 cycles, the fertility status information displayed each day on the monitor LCD and the days on which the study medications were taken.

It was not possible to retrieve monitor data in three study-cycles due to lost or broken devices, and there were no daily urine samples available from an additional cycle as the woman was abroad on holiday. Therefore, a total of 32 study cycles were analysed.

For the purpose of the study the following definitions were created.

Definitions based on the quantitative data.

A significant delay in the onset of next menses: Delay of 5 or more days from the expected onset of menses (based on the mean cycle length for the 2 placebo cycles).

The LH peak was defined as a significant rise in urinary LH concentration, with a minimum of 50% rise above the average baseline level for 3 preceding days and which remained elevated for a minimum of 3 days.

The first day of the LH peak was defined as the day of the first significant rise (>50% above the baseline) seen at the beginning of the LH peak.

Retrospectively predicted first day of the LH peak for the treatment cycles was the calculated mean of the first day of LH peak in the two placebo cycles.

A significant delay in the first day of the LH peak: Delay of 5 or more days from the expected first day of the LH peak in the treatment cycle (based on the mean first day of the LH peak during the 2 placebo cycles).

Luteal phase: time from the day after the first day of the urinary LH peak (LH +1) until, and including, the day before the first day of the next menses.

Follicular phase: time from the first day of the menses until the day of the first significant rise in urinary LH (LH +0) inclusive.

Definitions based on the monitor data. **High fertile days:** days preceding the urinary LH peak as indicated by the monitor to be potentially fertile. The monitor was

programmed to display an additional day of high fertility following the 2 peak fertile days.

Peak fertile days: The day of the first significant rise in urinary LH (>50% above the basal level over the preceding 3 days) detected by the monitor. The monitor was programmed to display a further day of peak fertility following the first rise.

4.2.3 Statistical Methods

The compliance data was summarised for descriptive purposes using numbers of cycles or tests as denominators.

5.2 Results

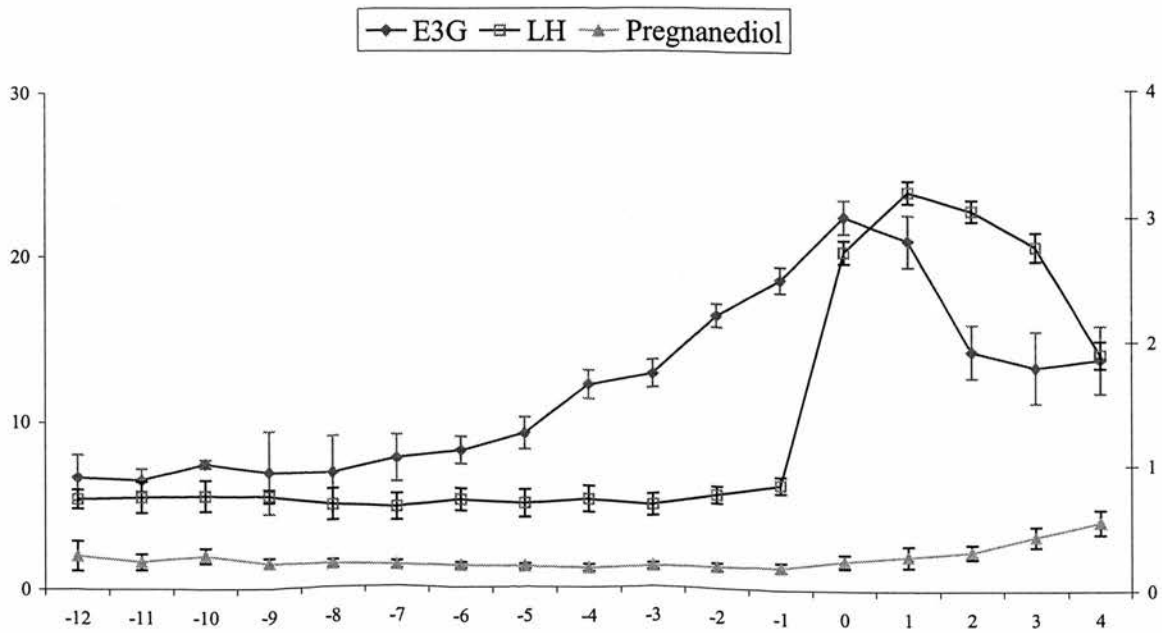
Data from the monitor and from RIA of daily urines was available in 32 study cycles, and in 11 of these (i.e. one treatment cycles per volunteer) women took LNG on a High fertility day. In the remaining 21 study cycles, placebo tablets were taken on a similar day. In four out of the 11 treatment cycles, LNG treatment aborted and or significantly delayed the subsequent LH surge. However, in the remaining seven treatment cycles, LNG did not alter the day of the LH surge or the cycle length significantly. In addition, one of the placebo cycles was prolonged due to a spontaneous delay in the LH surge. Therefore, the 5 cycles, in which the LH peak was significantly delayed in comparison to the usual pattern were analysed separately (Group 2) to the other 27 cycles (which consisted Group 1).

5.3.1 Group 1

Results from laboratory reference method for urinary hormones

The mean E3G levels in urine increased to maximum levels of 24.74 μmol/mol creatinine (95% C.I. 17.72 – 31.76), 1.2 days before the peak urinary LH value in the 27 ovulatory cycles. In these cycles, the mean concentration of peak urinary LH was 3.91 U/mol creatinine(95% C.I. 2.8 – 5.02) (Figure 5.1). As expected, the mean urinary P3G levels started to rise on LH +1.

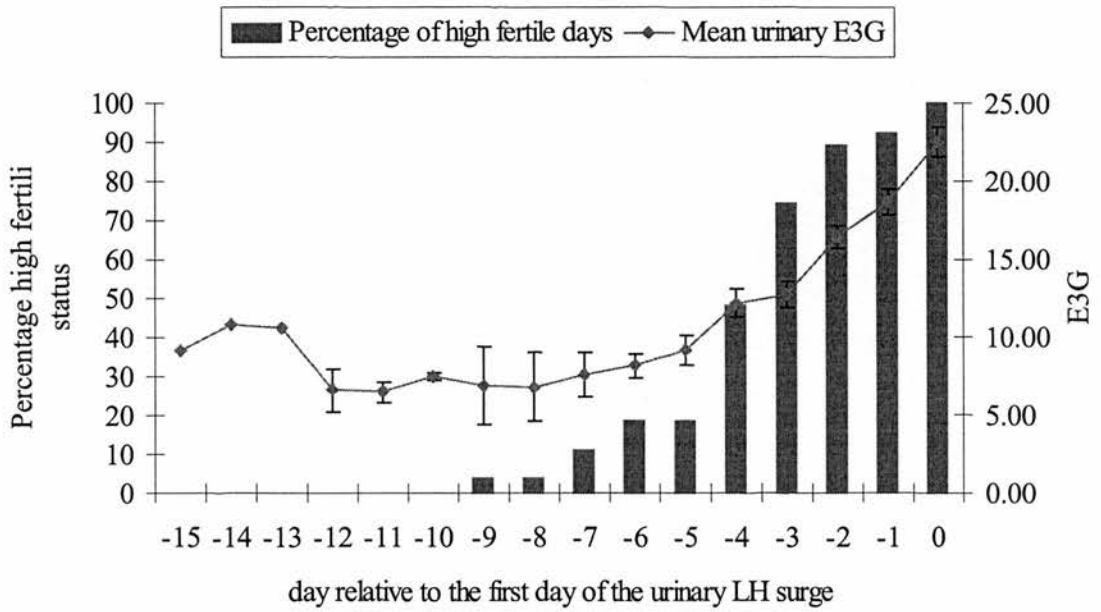
Figure 5.1 Mean urinary daily hormone levels by RIA



The monitor warning of the LH surge

The days of high fertility recognised by the monitor was regarded as the warning of the LH surge, and subsequent ovulation. It had been reported that the increase in E3G production from the enlarging follicle, with a parallel increase in serum levels and the corresponding urinary levels were associated with the monitor indicating increasing incidence of High fertility status on the days prior to ovulation (Behre *et al.* 2000). Among the 11 women, the monitor provided on average 3.4 days (95% C.I. 2.6 – 4.2; range 0 – 9 days) of warning prior to the first day of the urinary LH surge. In 74.07% of the cycles the monitor provided between 1 - 4 days of warning prior to the first day of the urinary LH surge (Figure 5.2).

Figure 5.2 Urinary E3G and percentage of high fertiity status



Comparison between the monitor peak fertility, and the urinary LH surge

The monitor detected an LH surge in 21 out of the 27 cycles in group 1. In all 21 cycles the monitor detected an LH surge within two days of the first day of the urinary LH surge measured by laboratory RIA (table 5.1, Figure 5.3). In these 21 cycles, the percentage rise in urinary (laboratory RIA) LH concentration above the baseline level (baseline value = average value of LH concentration during the preceding 3 days) was compared between the actual (laboratory RIA) LH peak day and the monitor peak day (Table 5.2). Except for 1 cycle, the daily urine samples analysed in the laboratory confirmed the LH percentage rise of > 50% above the baseline value on the day that the monitor declared an LH peak. In the remaining six cycles, an LH surge had occurred, but was not detected due to missed tests in one cycle; monitor error in two cycles; and could have been due to either of the above mentioned causes in three further cycles (no compliance data available for these three cycles).

Table 5.1 First day of the urinary LH surge (LH + 0) measured in the laboratory by RIA, relative to the first day of peak fertility indicated by the CPEFM monitor in group 1

Day of the LH surge by laboratory RIA of the daily urine samples relative to the monitor peak	***Frequency***	***Percentage %***
Before the first day of monitor peak fertility	3	14.3
On the same day as the first day of monitor peak fertility	16	76.2
After the first day of monitor peak fertility	1	4.8

Figure 5.3 Urinary LH and percentage of peak fertility status

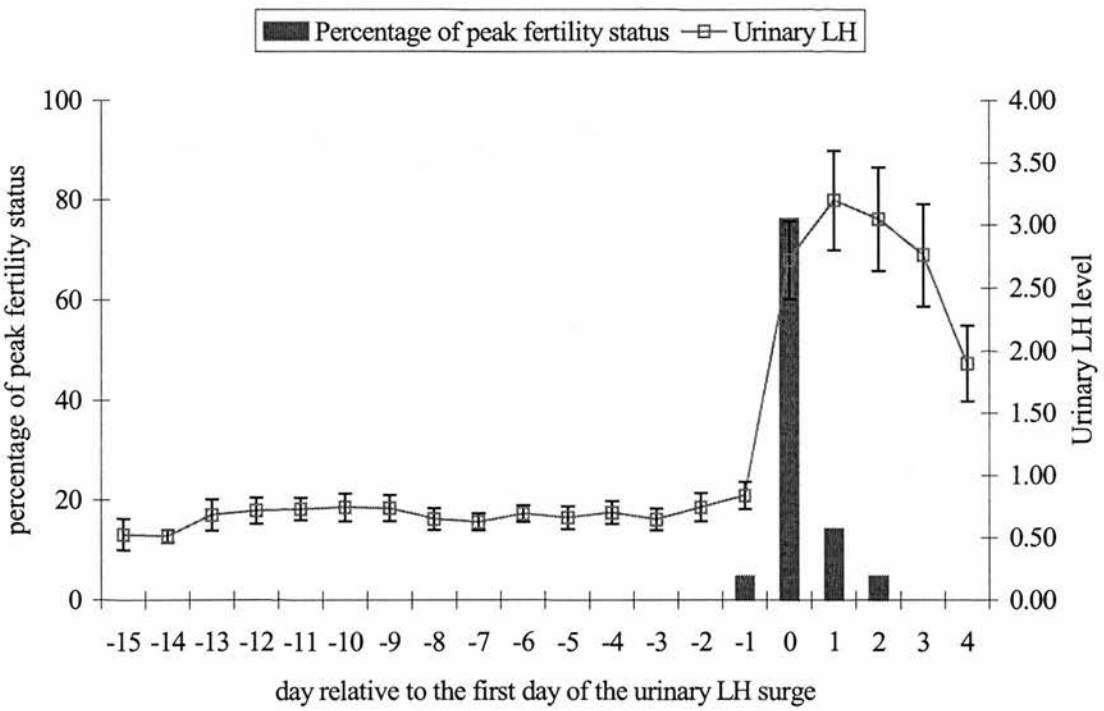


Table 5.2 The average percentage rise of the LH above the baseline concentration in collected daily urine (laboratory RIA) in group 1

The actual (laboratory RIA) LH peak	***The average % rise from the baseline on the day of the monitor peak***	***The average % rise from the baseline on the actual (lab-based urinary) LH surge***
Before the first day of monitor peak fertility (n=3)	226 (SD +/-108.0)	244.1 (SD +/-196.5)
On the same day as the first day of monitor peak fertility (n=16)	357.2 (SD +/-264.2)	357.2 (SD +/-264.2)
After the first day of monitor peak fertility (n=1)	13.64	105.13

5.3.2 Group 2

In these five cycles, the occurrence of a mid-cycle LH surge and subsequent ovulation, were either prevented or delayed (reflected by the absence of normal rise in urinary pregnanediol during the luteal phase). Nevertheless, a second E3G rise, a successive peak in urinary LH and a consequent rise in mid-luteal pregnanediol were observed later on, in the same cycle (Figure 5.4).

During these five cycles, at the expected time of ovulation for each woman the mean urinary LH reached peak concentrations of 1.1 U/mol creatinine (significantly lower than the value during the control cycles, $p < 0.0001$). The urinary E3G also increased to a peak on the same day (17.48 μmol/mol creatinine) but thereafter decreased down to an early follicular level. Furthermore, these lower than usual LH peaks were not followed by an increase in the urinary P3G. Subsequently, a second urinary LH peak (3.1 U/mol

creatinine, SD +/-1.0) occurred 17.4 days later in that same cycle (Figure 5.4), and was preceded by a peak in the mean urinary E3G level (19.06 μmol/mol creatinine SD +/-4.14).

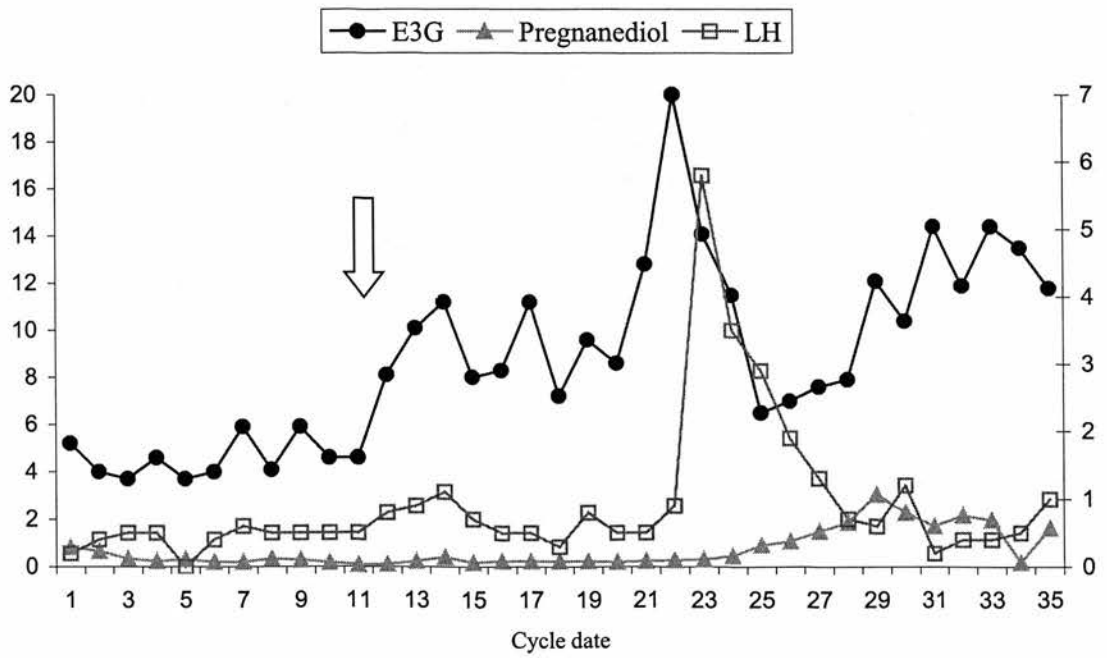


Figure 5.4 *Treatment cycle in subject S106.*

Note the delayed true LH peak on day 23 which is followed by an increase in the urinary pregnanediol levels. The white arrow represents the LNG administration.

Comparison between the monitor peak fertility, and the urinary LH surge

In three of these cycles, the monitor indicated peak fertility at the expected time for the respective volunteer (false positive). The monitor failed to identify every one of the second LH surges that occurred later in the cycles (between days 25 – 40). The reasons are as follows; In three cycles by the time the second LH surge had occurred, the monitor had ceased requesting tests; in one cycle the woman failed to perform a test requested by the monitor on the first day of the second LH surge (user error); in another cycle,

although a test was performed on the first day of the second surge, the monitor failed to identify it (monitor error) (Table 5.3).

Table 5.3 Reasons for missed LH surges in group 1 (27 cycles in total)

Reasons for the monitor not detecting an LH surge	***User Error (%)***	***Monitor error (%)***	***Unknown***
Number of cycles	1	2	3
Percentage of the total number of cycles	3.7%	7.4%	11.1%

5.3.3 Objective measurement of compliance with urine testing

Compliance data on daily urine testing was available in 26 cycles, and this information could not be retrieved from the monitors in 6 cycles due to infrequent downloading. The eleven women failed to perform 18.9 % (95% C.I. 16.7 – 25.1) of the total tests requested by the monitor. On average a woman missed between 2 – 3 tests of the average 12.2 tests requested (range 10 – 20 tests).

5.4 Discussion

A reliable and simple means to predict the potentially fertile period will provide essential information that can be used to influence the probability of conception. If the users can be reliably warned of the potentially fertile period in each cycle, that information can be employed in clinical practice, to time intercourse in order to avoid or permit the occurrence of a conception as desired. Such methods will assist in contraceptive research to monitor the frequently changing hormone levels, and to identify the fertile period.

Hormonal methods of EC are widely used to prevent pregnancy after unprotected coitus (Glasier 1997 review). Sex steroids that are used as ECs have several effects on both the ovulatory cycle and on the endometrium. These effects are dependent on the time in the

cycle that the EC is administered. Therefore, the accurate prediction of the potentially fertile period should enable investigators to identify the particular mechanism of the sex steroid that prevents conception when intercourse has already taken place.

The precision of the method that identifies the fertile period directly affects the accuracy of the estimated probability of conception following an act of unprotected intercourse. Therefore, one can see that the accuracy of the estimated efficacy of any EC method is directly dependent on the technique used to identify the fertile period.

Previous studies have confirmed that the home-use fertility monitor data correlated well with the laboratory measured urine hormone levels (Bonnar *et al.* 1999; Behre *et al.* 2000). In contrast to the laboratory assays that are inconvenient to women (e.g. collection of a large number of urine samples), time consuming, and expensive, the monitor was rather easy to use and was possibly less expensive.

The consensus of opinion is that the fertile period of the woman's cycle is around six days, ending on the estimated day of ovulation (Wilcox *et al.* 1995). A peak in urinary LH levels preceded ovulation by about 24 hours (Collins *et al.* 1996). In our study, to test the hypothesis that LNG acts as a post-coital agent by abolishing the pre-ovulatory LH surge and by delaying ovulation, we wanted to administer LNG in the fertile period immediately before the mid-cycle LH surge. Therefore, we required in particular, the prediction of the impending LH surge to be accurate (the warning had to be at least one day, but not more than four days before the urinary LH surge), to administer LNG at a matching time in each cycle in the fertile period, so the observed effects could be compared. The correlation between the High and Peak fertility days as indicated by the monitor and the urinary LH surge, showed (Figure 5.2) that in 25.93% of the cycles in group 1, there was either no warning or the warning started at more than four days before the LH peak had occurred. When the treatment had to be administered on the actual first day of the LH peak, it created a problem when comparing the effects of LNG taken on or

before the day of the urinary peak. It is possible that the timing of LNG in the former group was "too late" to influence an event already well underway. Similar obstacles were encountered in data analysis when LNG was administered long before ovulation had occurred (as a result of the advance warning of the imminent occurrence of an LH surge more than four days before the actual surge). The Clearplan Easy device is designed for a different purpose (for couples intending conception therefore the users should continue to have intercourse until peak fertility is recorded) hence "the prolonged fertile period does not matter" (Bhiwandiwalla *et al.* 2001) is not a valid argument for the following reasons. The purpose of using the device is to time intercourse since not all couples are able to have intercourse every day – indeed, if they were, there would be no need to monitor ovulation. If users exhaust themselves by having intercourse for several days before the days of peak fertility, they may be less likely to have intercourse at a time when the chance of pregnancy is maximal.

We have previously reported a monitor error of 7.9% in identifying an LH surge (Chapter 3; Hapangama *et al.* 2001b), and a similar performance with about 92% accuracy in LH detection was expected in this group of patients. Therefore, non-compliance with urine testing was likely to be the cause for missing the LH surge in the four out of the six cycles in group 1. Women missing about 19% of the urine tests requested by the monitor is obviously significant and comparable to previous observations by our group (Chapter 4; Hapangama *et al.* 2001c). The monitor stored data simultaneously on the hormone signal levels and on the testing events (volunteer compliance) for several months and this information could be later retrieved. Among urine samples that have been collected daily over a period, a wrongly labeled one will be impossible to detect, yet may affect the results. In addition, self-LH detection kits have been commonly used to identify the LH surge, for example to time the administration of a particular agent, and to sample the endometrium at a particular time (Cameron *et al.* 1996). Obviously, inaccuracy in detecting the LH surge (or undetected non-compliance) in such studies directly affects and invalidates the results. Therefore, the monitor offered

us an important methodological advance in providing reliable data on the incidence of non-compliance with urine testing, and exactly when a test was missed.

Several possible improvements could be made to the system to achieve its full potential use in clinical research studies. Although the urinary LH surge detection based on a significant rise preceded by a similar rise in E3G may be adequate for most of the normal cycles, it will erroneously detect an LH peak that had been aborted. If the qualifying criteria included the rise in LH to remain elevated for a minimum of 3 days, and if the system revalidated the declared LH surge, for example three days later, on the basis of the sustained increase in urinary LH signal and on the rise in P3G, the monitor accuracy will be significantly enhanced. Similarly, if the monitor requested daily urine tests throughout the cycle, the delayed LH surges may have been identified. The daily requirement in testing may even increase the overall compliance since the user may get into a habit of performing the test every day.

This study was designed to assess the pragmatic issue of whether the home-use fertility monitor can reliably replace the existing daily urine and whether it offers increased convenience to women. In a significant number of cycles the monitor indicated high fertility long before the ovulation had occurred and thus, there is really no advantage to providing this advance warning either in a clinical or a research setting. Conversely, as the rise in LH is the most predictable method of determining the days of high fertility (WHO 1980), an error of just around 8% in LH detection can be tolerated. In conclusion, this microelectronic system in detecting the urinary LH peak can be invaluable in contraceptive research because it is easy to use and provides reliable data on compliance. However, further technical adjustments are called for in giving advanced warning of the fertile period (identifying High fertile days).

CHAPTER 6

THE ENDOMETRIAL EFFECTS OF MID-LUTEAL PHASE ADMINISTRATION OF MIFEPRISTONE

6.1 Introduction

Human endometrium is a target organ for the ovarian steroidal hormones oestradiol and progesterone. One of the fundamental roles of progesterone is the differentiation of an oestrogen-primed endometrium (Corner & Allen 1929). The endometrial receptivity that permits successful implantation depends upon timed and regulated synthesis and secretion of a specific set of progesterone-induced proteins in an oestrogen-primed endometrium. If a pregnancy fails to occur, the corpus luteum regresses with a subsequent fall in progesterone levels. There is now compelling evidence that a period of exposure of the oestrogen-primed endometrium to progesterone, followed by a withdrawal of progesterone are the hormonal prerequisites for menstruation (Critchley *et al.* 2001). The characteristics of the endometrial changes (including the extensive changes observed in the endometrial vasculature) associated with the withdrawal of progesterone and menstrual bleeding, suggest an involvement of vaso-active local mediators. Prostaglandin (PG) activity in the endometrium is modulated by progesterone, and their widely recognised vasoactive properties make PGs to be prime candidates for mediators of progesterone action on the endometrium (Baird *et al.* 1996).

Prostaglandins (PGs) are synthesised from arachidonic acid (AA) thus, the liberation of AA from precursors (present as membrane bound phospholipids) by the action of phospholipase A_2 (PLA_2) is one of the first and a rate limiting steps in PG synthesis (Bonney *et al.* 1987 a & b; reviewed in Poyser 1992). AA is then converted to prostanoids including PGE_2, $PGF_{2\alpha}$, and PGI_2 by the actions of cyclo-oxygenase (Figure 6.1). In the endometrium, PGs are not stored, but immediately synthesised and released, and metabolised to inactive metabolites by prostaglandin 15 dehydrogenase (PGDH) enzyme (Ensor *et al.* 1990).

The antiprogesterone compound mifepristone (RU 486; Roussel Uclaf, Paris, France) is a synthetic 19-norsteroid with a specific high affinity for binding to the progesterone receptor. It blocks the biological effects of progesterone at the level of the progesterone

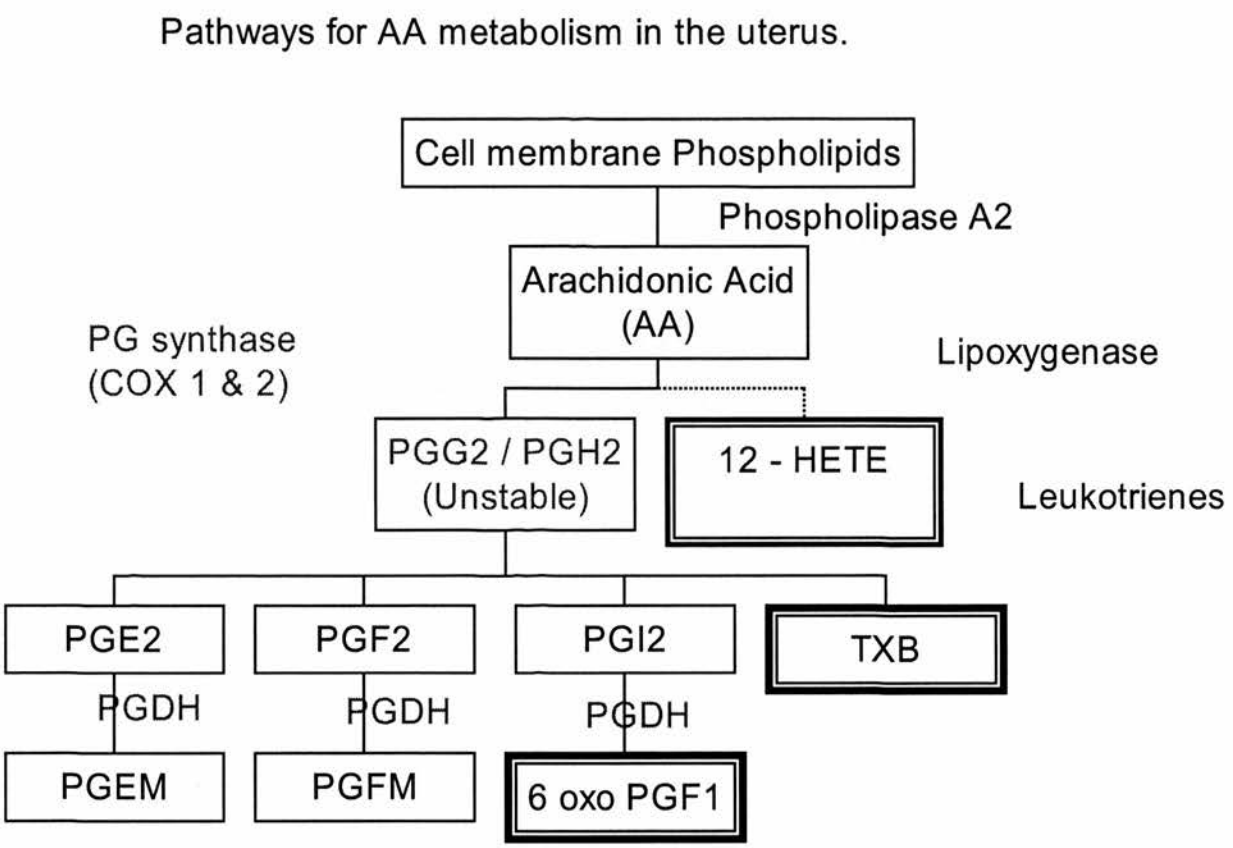


Figure 6.1 *Pathway of Arachidonic acid metabolism in the Uterus*

Predominating in: endometrium myometrium

receptor (Spitz & Bardin 1993). In the mid-luteal phase, mifepristone at a single dose of 50 - 800 mg, induces menstrual bleeding within 72 hours (Schaison *et al.* 1985; Shoupe *et al.* 1987). Luteolysis was incomplete in two thirds of the subjects, and they experienced a further episode of vaginal bleeding at the expected time of menses. In the remainder there was complete luteolysis with only one episode of bleeding. Thus the vaginal bleeding observed after mifepristone without a decrease in the circulating progesterone values, appears to be due to a direct effect on the endometrium. Johannisson *et al.* in 1988 suggested that the menstrual bleeding induced by mifepristone in mid-luteal phase to be a direct endometrial vascular effect. Following treatment with 50 mg of mifepristone in the mid luteal phase (days 20 - 23), they

described a significant reduction in the capillary luminal area and diameter associated with degenerative changes in the endothelial cells, which preceded the menstrual shedding. These changes did not always accompany regressive changes in the adjacent stroma. This effect of mifepristone on the endometrium (at a time when the endometrial progesterone receptor level is relatively low) is poorly understood, and has been hardly investigated.

The endometrial effects of early luteal phase administration of antiprogesterones have been extensively investigated (Swahn *et al.* 1990; Gemzell-Danielsson *et al.* 1996; Cameron *et al.* 1996). In the early luteal phase mifepristone inhibits progesterone induced down-regulation of PR and oestrogen receptors while antagonising the progesterone action on endometrial markers such as prostaglandin dehydrogenase (PGDH), which are known to be progesterone dependent (Casey *et al.* 1980; Greenland *et al.* 2000). Moreover, PGDH has been postulated as a useful marker of the "closure" of the implantation window, and the effect of mid-luteal administration on such markers might add to our current understanding of potential contraceptive actions of mifepristone.

Our study investigated the mechanism of mifepristone-induced endometrial shedding & vaginal bleeding in the endometria of 16 healthy women with regular cycles. The endometrial biopsies were performed between 0 (control), and 6 to 48 hours following mid-luteal phase administration of a single dose 200 mg of mifepristone. We examined the expression and the distribution of sex steroid receptors in the endometrium and the expression of PGDH, and cyclo-oxygenase – 2 (COX-2). Alterations in the expression of such progesterone-dependent proteins may widen our understanding of the mechanism by which mifepristone induces endometrial bleeding in the mid-luteal phase.

6.2 Materials & Methods

6.2.1 Subjects

Initially, 20 healthy women with regular cycles (25 - 30 days duration) aged between 26 and 42 years (mean age 34 years) were recruited in to a randomised, single centre study with mifepristone. Women were either using a reliable non-hormonal method of contraception or were abstinent. All women underwent a comprehensive screening procedure before commencing the study. This consisted of a full medical history and routine physical and gynaecological examination, together with measurement of blood pressure, pulse, height and weight. In addition, a venous blood sample was taken for full blood count, serum biochemistry and liver function. These blood tests were repeated at the end of the study. All women kept a menstrual diary card, and recorded all vaginal bleeding experienced during the study period and in the following cycle, and the day in which they identified an LH surge using urinary dipsticks.

We also studied the endometrial samples from four women taking part in a separate study, which evaluated the secretory endometrium. Those women also used the same urinary dipsticks to identify the urinary LH surge and the biopsies were collected 7 or 8 days after the first day of the urinary LH surge. Since these women did not receive any treatment the four biopsies were included in to our control group. Therefore, the total number of biopsies analysed in the control group was seven. Lothian Research Ethics Committee approved the study and, informed written consent was obtained from each woman.

6.2.2 Study Design

The women were monitored over two consecutive cycles; a treatment and follow-up cycle. Each of the 20 women in the study were allocated to the next consecutive study number in the randomisation list before commencing the study. The randomisation list was produced using SPSS such that each study number was randomly assigned to one of five groups in the study. The list was balanced after each block of 5.

Women used LH detection kits (Oviquick, Unipath, Bedford, UK) to detect the Luteinizing Hormone surge in a first sample of urine.16 women took 200mg of mifepristone on day 8 after the onset of the urinary luteinizing hormone surge (LH+8). An endometrial biopsy was obtained at 6, 24, 36 or 48 hours after taking mifepristone. In order to assess the steroid receptor expression and endometrial parameters of the normal secretory endometrium in the mid-luteal phase, four women were randomly allocated to a control group. They did not take mifepristone, but had an endometrial biopsy 8 days following the identification of the LH surge. On the occasion when the endometrial biopsy was taken, a blood sample was also collected for serum progesterone measurement by radio-immunoassay (See Chapter 7).

Four women were subsequently withdrawn from the study. One due to previously undiagnosed cervical stenosis; in one woman RIA could not confirm the self-detected LH peak; the endometrial samples were inadequate for analysis in two other women. Therefore, we analysed the endometrial samples in 13 women after taking mifepristone in the mid-luteal phase (n = 3, at 6 hours; n = 4, at 24 hours; n = 3, at 36 hours; n = 3, at 48 hours; after mifepristone). Three samples from our original control group, and the above mentioned four additional samples from a separate study made the total number in the control group to be 7.

6.2.3 Detection of the urinary LH peak

The timing of the urinary LH surge were detected by the subjects themselves using a commercially available LH detection kit (Oviquick, Unipath, Bedford, UK), which they used according to the manufacturer's instructions. Self-detected urinary LH peak was subsequently confirmed by radio-immunoassay (See Chapter 7 - Methods).

6.2.4 Serum progesterone

A blood sample was collected immediately before the endometrial biopsy in all women, stored, and later assayed for progesterone level (methods - Chapter 7). Serum

progesterone measurements were done by using the Coat-A-Cont progesterone procedure (a solid-phase Radio Immuno Assay).

6.2.5 Endometrial biopsies

Endometrial biopsies were obtained using a Pipelle endometrial sampling device (Prodimed, Neuilly-en-Thelle, France) without the need for cervical dilatation or general anaesthetic and were fixed immediately in 4% para-formaldehyde for 24 hours, routinely processed, embedded in paraffin, and sections were cut to 5 μm thickness. All tissue samples were labelled with a code number for anonymity, and except for this number, the mounted sections did not contain any other information.

6.2.5 Immunohistochemistry

Immuno-histochemical staining was performed for immuno-localisation of:

i) Progesterone receptor (PR) with mouse monoclonal anti-human PR antibody (Novocastra Laboratories, Newcastle upon Tyne, UK).

ii) Oestrogen receptor (ER) with mouse monoclonal anti-human ER antibody ER1D5 (DAKO Laboratories, High Wycombe, UK).

iii) Androgen receptor (AR) with monoclonal mouse anti-human AR antibody (F-39, Biogenex antibody, A Merarini Diagnostics, UK).

iv) Prostaglandin dehydrogenase (PGDH) with rabbit polyclonal antibody (Dr H.H.Tai, University of Kentuckey, Lexington, USA).

v) Cyclo-oxygenase type –2 (COX-2) with goat polyclonal anti-human COX-2 antibody (Santa Cruz, Biotechnology, UK).

Immunohistochemistry procedures

All protocols were optimised to determine the correct conditions for maximum specific staining, and all sections used as negative controls did not show immunostaining. Each immunostaining procedure was performed in a single run.

(Chapter 7 – Methods).

6.2.6 Scoring and immunohistochemistry analysis

We employed a semi-quantitative subjective scoring system to evaluate the intensity and the localization of immunoreactivity in entire tissue sections. Previously we have reported that the immunostaining patterns in endometrial sections measured by the subjective semi-quantitative scoring, showed an almost perfect correlation (regression coefficient of 0.963) with that measured objectively by computerized image analysis (Wang *et al.* 1998). Therefore, the less time consuming, semi-quantitative scoring system provides a valid score suitable for graphical presentation.

Two independent observers using light microscopy visually assessed all coded sections. The two separate scores were then compared to obtain a more objective final score for each section. Once the final score had been agreed for all sections in the five-immunostaining runs, the code was broken. Afterwards the final immuno-staining scores were analysed by the respective groups.

The immuno-staining intensity of the steroid receptors (PR, ER, and AR) were scored using a four point scoring scale, where the intensity of staining was assigned as 0 = none, 1 = weak, 2 = distinct, 3 = strong. However, the staining intensity of PGDH and COX-2 showed a narrow range and therefore we adapted a three point scoring scale where the score of zero indicated an absence of immuno-reactivity; 1 = faint immuno-reactivity; 2 = strong immuno-reactivity.

6.2.7 Statistical analysis

Since mifepristone induced bleeding by 36 – 48 hours in all women, a preliminary analysis was performed to determine the appropriate statistical test for analysis of the data. The mean staining intensity scores between 6 hour and 24 hour groups and between 36 hour and 48 hour groups showed no significant difference, hence the 13 samples after treatment with mifepristone were analysed in two groups; group 1 (6 to 24 hours after mifepristone n = 7) and group 2 (36 to 48 hours after mifepristone n = 6). Comparisons

between these two groups, was tested by non-parametric Kruskal-Wallis ANOVA test, and the Dunn's Multiple Comparisons Test since they were discontinuous data sets.

6.3 Results

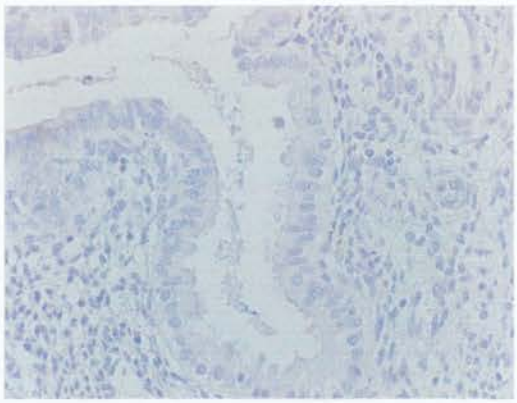
All women reported vaginal bleeding commencing 36 – 48 hours after taking mifepristone. Four women in group 2 (one woman after 36 hours, and three women after 48 hours) had already started to bleed at the time the endometrial biopsy was taken, the others started bleeding after the biopsy. The bleeding lasted for 12 – 72 hours, and all but three women reported a second bleed at the time of the expected menses. In these three women, a second episode of bleeding occurred approximately four weeks later.

6.3.1 Serum Progesterone

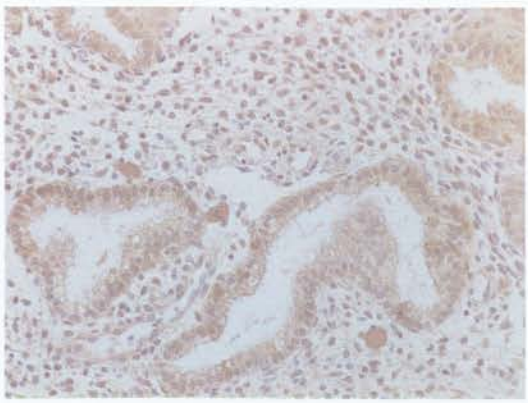
Treatment with mifepristone had significantly reduced serum progesterone levels in all women when compared with the controls (13.2 nmol/L vs. 34.8 nmol/L, $p = 0.001$), but the levels between 6-24 h group and 36-48 h group were not statistically significant ($p = 0.32$).

6.3.2 PGDH immunoreactivity

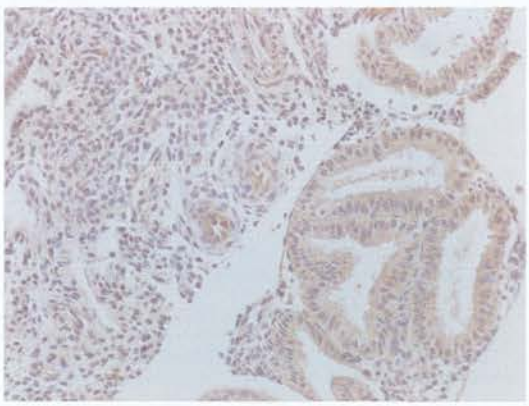
Intense PGDH immunoreactivity was observed in the cytoplasm of predominantly glandular epithelium (with a lesser degree of staining in the stromal cells) in all mid-luteal phase control sections. The abundance of PGDH positive immunostaining clearly declined to be virtually absent by 36 - 48 hours in both glands and in stroma. The difference in PGDH staining scores between the control group and 36 - 48 h group were significant ($p = <0.05$) (Figures 6.2 and 6.3).



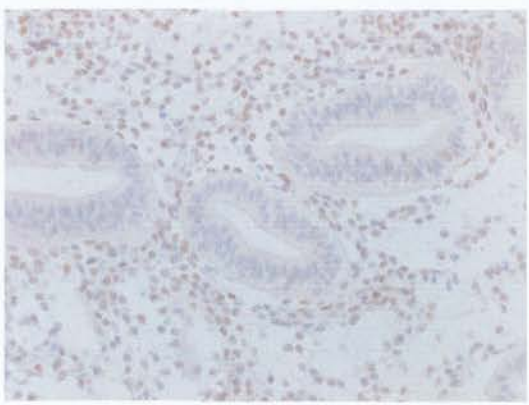
a. Negative control



b. Control (0h) sample on LH+8



b. 6 – 24h after mifepristone



c. 36 – 48 h after mifepristone

Figure 6.2 PGDH immuno-staining

a. Negative control = Mid-luteal endometrium.
b. PGDH immuno-staining in midluteal phase endometrium, demonstrating positive immuno-reactivity in the glands and stroma.
c. Endometrium biopsied 6-24h after mifepristone, showing mild decrease in immunostaining in both compartments.
d. Note the clear decrease in immuno-staining in both glandular and stromal compartments of the endometrium biopsied 48h after taking mifepristone on LH+8.

Scale bar, 50 μm.

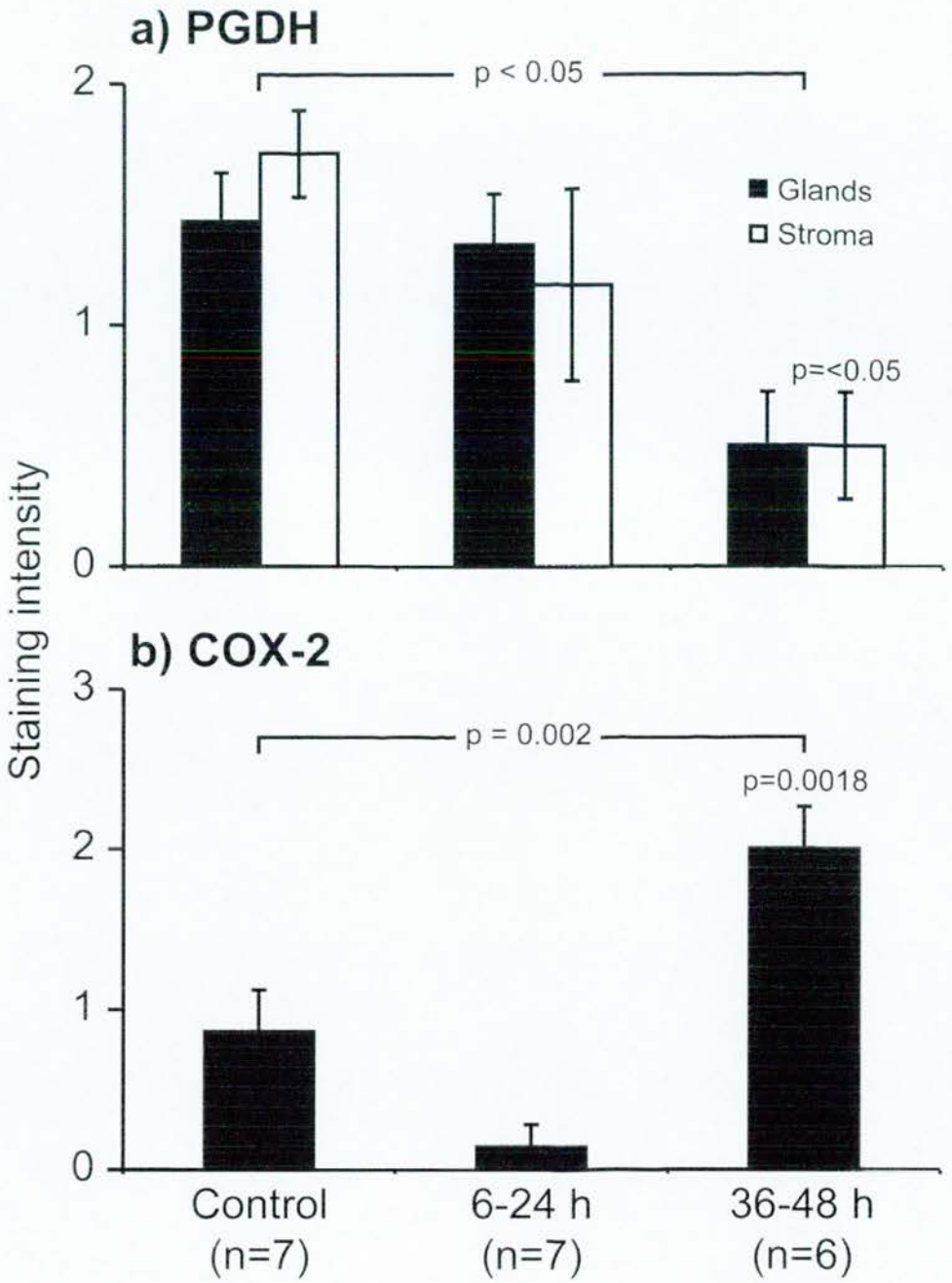
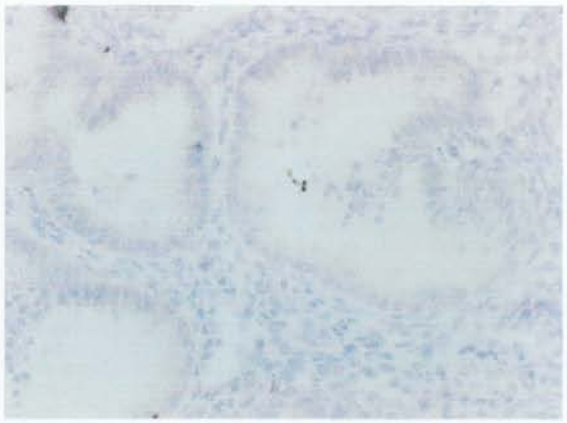


Figure 6.3

Intensity of staining for PGDH (a) and COX-2 (b) in endometrial glands (■) and stroma (□) in the mid-luteal phase of the cycle before (control) and at times after administration of 200mg mifepristone.

Bar, Median and range



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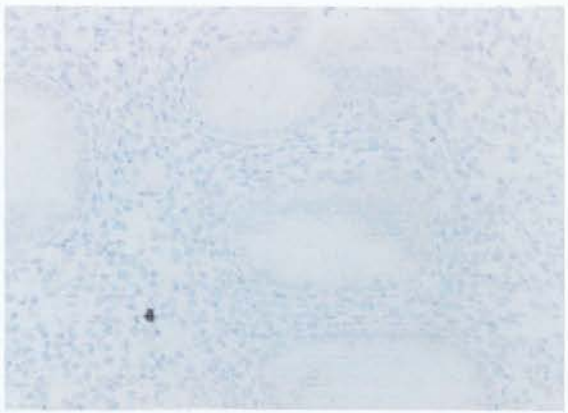
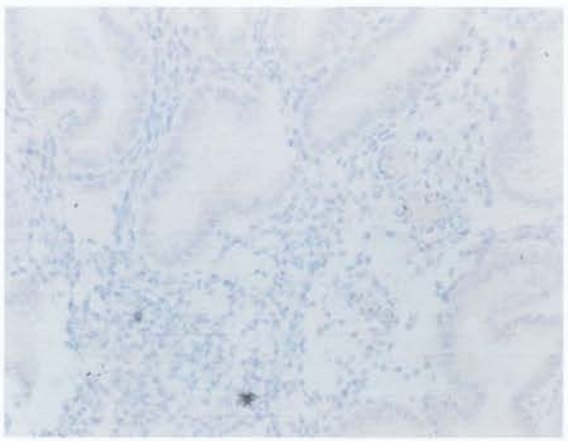
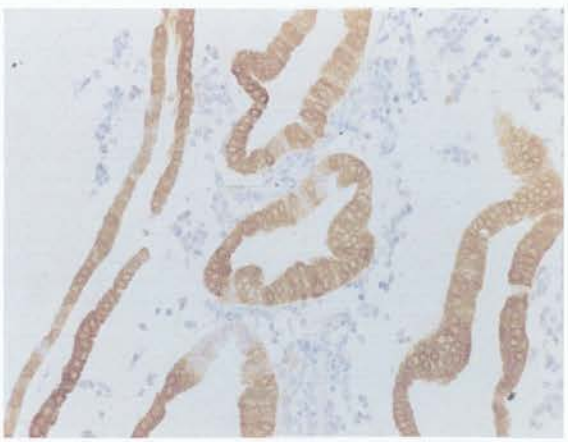


b. Control (0 h) sample on LH+8



c. 6 – 24 h after mifepristone

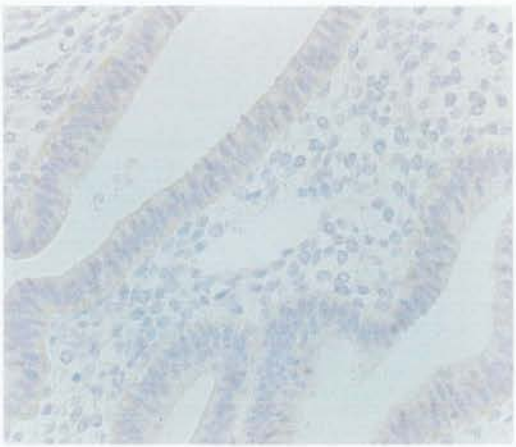


d. 36 – 48h after mifepristone

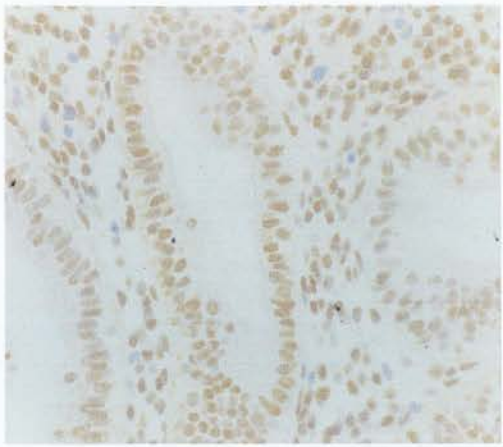
Figure 6.4 COX-2 Immunostaining

a. Negative control = mid-luteal endometrium.
b. COX-2 immuno-staining in an endometrium collected in the mid-luteal phase illustrating negligible immuno-staining in both glands & stroma.
c. No obvious change in the endometria for COX-2 immuno-reactivity biopsied 6-24h following mifepristone.
d. Endometrium biopsied 36-48h after mifepristone, on LH+8 showing a marked increase in immuno-staining in the glandular cytoplasm.

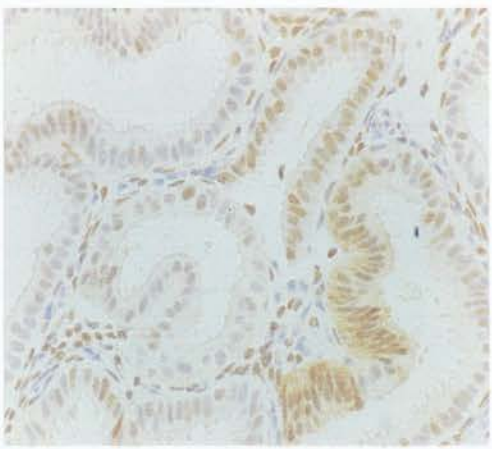
Scale bar, 50 μm.



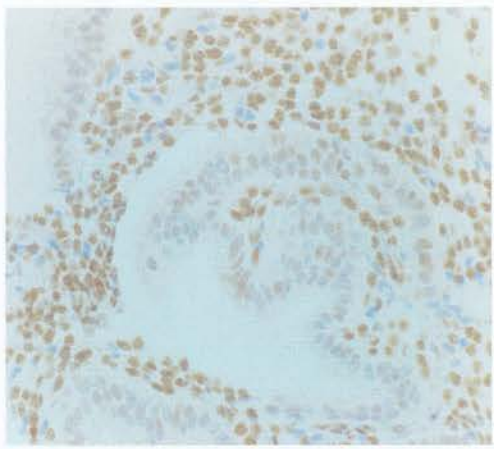
a. Negative Control



b. Control (0h) sample LH+8



c. 6 - 24 hours after mifepristone



d. 36-48 h after mifepristone

Figure 6.5 PR immuno-staining

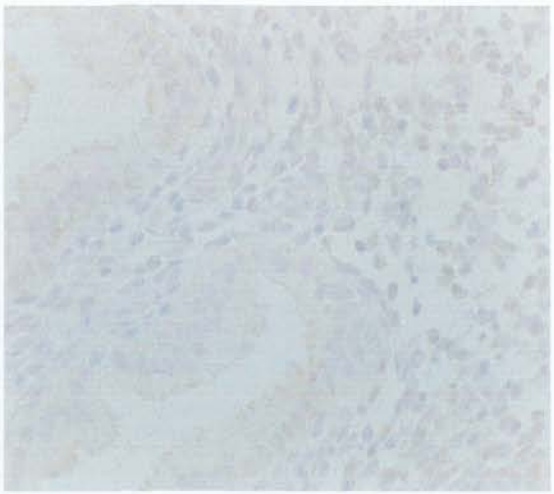
a. Mid-luteal endometrium, negative control.

b. A control biopsy displaying faintly positive PR immuno-reactivity confined to the nuclei of both glandular epithelial and stromal endometrial cells in the mid-luteal phase (LH+8) endometrium.

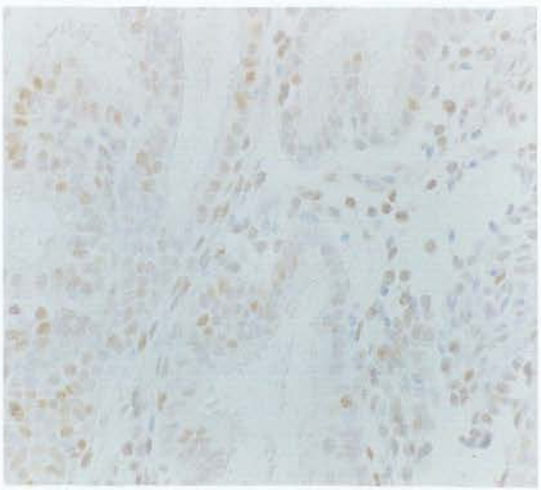
c. The weak glandular PR staining in an endometrial sample biopsied 6-24h after mifepristone.

d. Glandular PR immuno-staining appears to decline further by 36 - 48 hour after mifepristone. Note the persistence of the weak stromal staining across the groups.

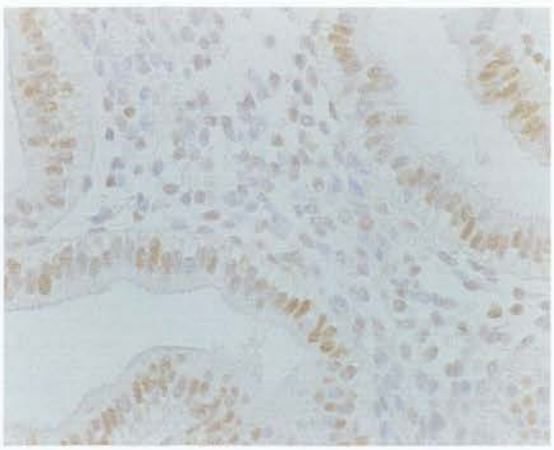
Scale bar, 50 μm.



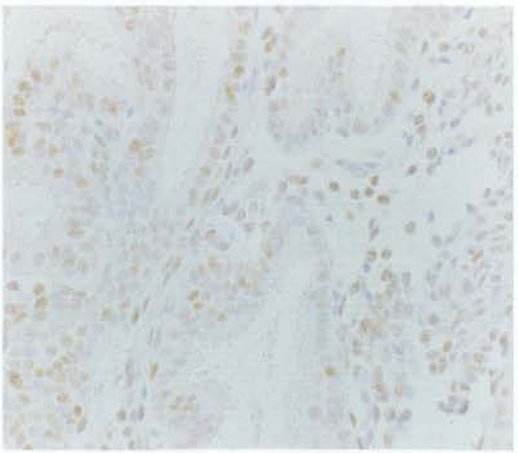
a. Negative control



b. Control (0 h) sample at LH+8



c. 6 –24 h after mifepristone



d. 36-48h after mifepristone

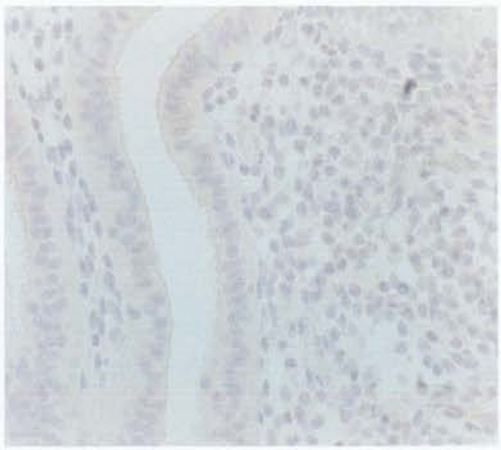
Figure 6.6 ***ER immuno-staining***

a. Negative control = mid-luteal endometrium.

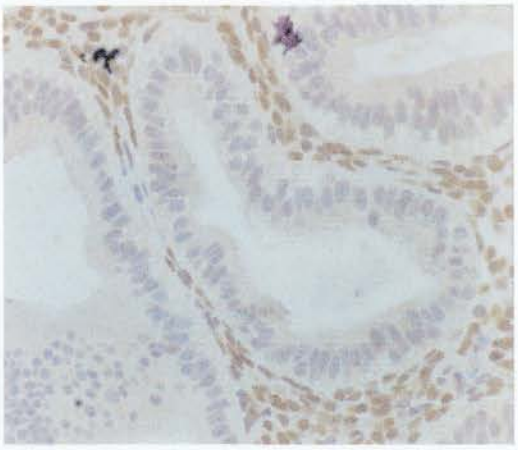
b. Mid-luteal phase control sample, showing weak ER immunostaining in the stroma as well as in the glands.

c. & d. Endometria biopsied 6-24h and 36 – 48h after mifepristone, respectively, showing no obvious change in the staining.

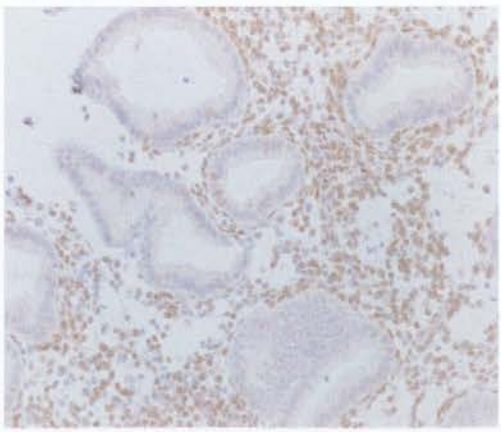
Scale bar, 50 μm.



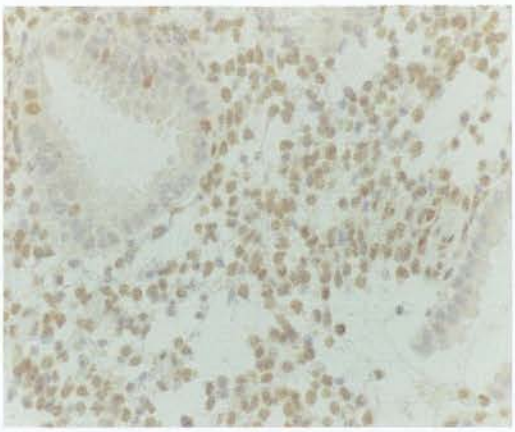
a. Negative control



b. 0 hours (Control) sample on LH+8



c. 6 –24 h after mifepristone



d. 36 – 48 h after mifepristone

Figure 6.7 Androgen receptor immuno-staining

a. Negative control = Mid-luteal endometrium.

b. Control endometrium biopsied on LH+8, showing the distinct AR immunostaining localized predominantly in the stroma with faint or absent glandular epithelial staining.

c. & d. Endometria biopsied after mifepristone showing no apparent change in the AR immuno-staining.

Scale bar, 50 μm.

6.3.3 COX-2 immunoreactivity

In all samples (treated / untreated), COX-2 staining was localised predominantly in the endometrial glands with no (or barely detectable) staining in the stroma (Figure 6.4). Untreated endometria in the mid-luteal phase showed minimal staining for COX-2 in the glandular cellular compartments. After mifepristone, a significant increase in immunoreactivity was apparent at 36 - 48 hours (Figure 6.3) ($p = 0.0018$).

6.3.4 Steroid receptor immunostaining.

Control biopsies displayed faintly positive PR immuno-reactivity confined to the nuclei of both glandular epithelial and stromal endometrial cells. The weak glandular PR staining appears to decline further by 36 - 48 hour after mifepristone. Stromal staining however remains weak across the groups (Figure 6.5).

In all mid-luteal phase control samples, there was weak ER immunostaining in the stroma as well as in the glands. There was no obvious change in the staining after mifepristone (Figure 6.6).

The distinct AR immunostaining was localized predominantly in the stroma in the control samples with faint or absent glandular epithelial staining, and there was no apparent change observed in the AR immunostaining after the treatment with mifepristone (Figure 6.7).

6.4 Discussion

Endometrial shedding and vaginal bleeding is observed after withdrawal of progesterone (e.g. luteal regression) from an oestrogen primed endometrium that is subsequently exposed to progesterone. Similar bleeding is also seen after the pharmacological withdrawal of progesterone via administering the antiprogesterone, mifepristone in the luteal phase of the cycle. While the endometrial morphology exhibits a marked sensitivity to mifepristone, with 0.5 mg daily dose being the threshold dose for delay in

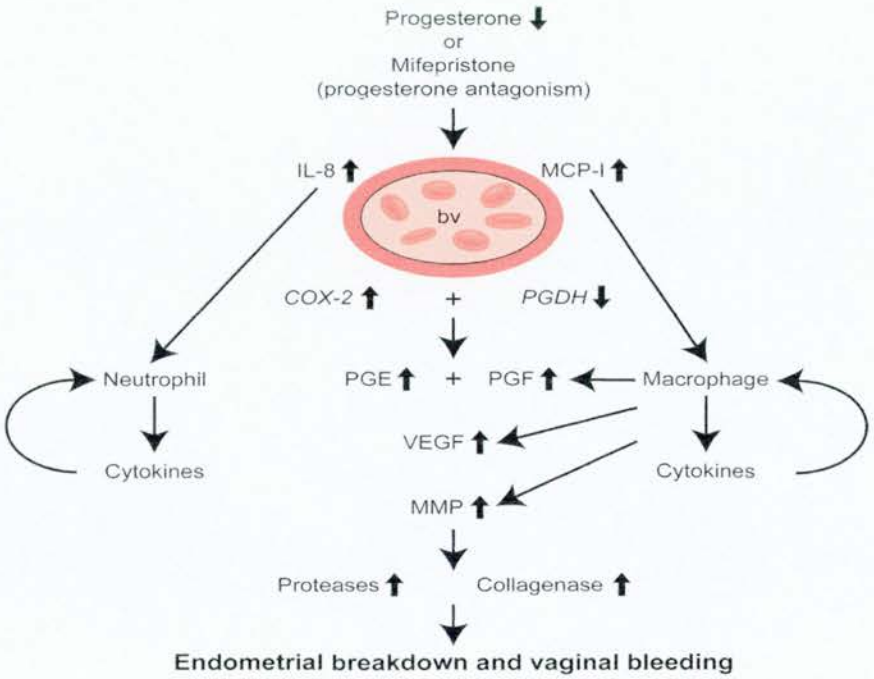
endometrial maturation (Croxatto *et al.* 1993; Cameron *et al.* 1996; Spitz *et al.* 1996; Gemzell-Danielsson *et al.* 1997), in general, higher doses (in excess of 10 mg) are required to produce endometrial shedding and menstrual bleeding (Green *et al.* 1992; Schaison *et al.* 1985; Shoupe *et al.* 1987).

All women in our study reported vaginal bleeding commencing at 24 to 48 hours after taking 200 mg of mifepristone in the mid-luteal phase. The mifepristone induced bleeding in the mid-luteal phase, was not dependent on a parallel decline in the circulating progesterone levels, but was due to direct antagonism of the action of progesterone on the endometrium (Swahn *et al.*1988). The endometrial shedding at the onset of normal menstruation is due to a fall in progesterone levels that follow luteal regression. In 13 out of the 16 subjects, this was followed by a second bleed of normal character at the expected time of the next menses. Although mifepristone significantly depressed the serum progesterone value in all women, the occurrence of a second bleed in the majority suggest only a partial luteolysis and a direct effect of mifepristone on the endometrium.

The current understanding advocates a central role for PGs as a trigger mechanism for menstruation (Figure 6.8, Baird *et al.* 1996; Critchley *et al.* 2001; Milne *et al.* 2001). The concentration of PG in any tissue is related to its rate of synthesis and metabolism, and the variation in the endometrial release of PGs at different stages of the menstrual cycle suggests an ovarian hormonal influence. The two key-enzymes that control the endometrial PG synthesis (PLA_2 and COX) appear to be under the influence of progesterone. COX enzyme exists as two isoforms produced by two different genes (COX-1 and COX-2). COX-I is constitutively expressed while COX-2 expression is modulated by a variety of stimuli and may be inhibited by progesterone in the endometrium (Jones *et al.*1997). PLA_2 also appears to be present in the endometrium in two different iso-forms; the calcium dependent and inducible $PLA_{2(i)}$ which is localised in the endometrial glands, and the calcium independent $PLA_{2(ii)}$ that is predominantly

confined to the stroma (Bonney *et al.* 1987). Conversely, prostaglandin dehydrogenase (PGDH) metabolises PGs to inactive metabolites and this enzyme is induced by progesterone (Casey *et al.* 1980 & 1993; Kelly *et al.* 1986). The increase in PGDH activity in a secretory endometrium that occurs in response to the rising levels of progesterone in the luteal phase (Casey & MacDonald 1980) can be prevented by administration of antigestagens shortly after ovulation (Cameron *et al.* 1997). Thus progesterone is responsible for stimulating PGDH and suppressing COX-2.

Figure 6.8 *Vascular origins of endometrial shedding & vaginal bleeding*



The decline in progesterone concentrations (luteal regression) or inhibition of its action (mifepristone) exerts a primary effects on the peri-vascular region surrounding blood vessels (bv). Adapted from; Baird *et al.* (2000) with permission.

In our study, treatment with mifepristone in the mid-luteal phase resulted in a decreased PGDH and an increased COX-2 expression in the endometrial glands, which was apparent at 36 hours following mifepristone. This effect will be synergistic in increasing

endometrial PGs (due to a synchronized suppression of metabolism, with an augmentation in the synthesis), and will lead to increased uterine activity and menstrual bleeding. In addition, progesterone withdrawal mediates the degradation of the endometrial extra-cellular matrix by inducing matrix metalloproteinases (MMP) (Lockwood *et al.* 1998), and other cytokines (Critchley *et al.* 2001 & references therein).

Attempts have been made to demonstrate the effects of progesterone on the endometrial PGs activity both in-vitro as well as in-vivo studies. Progesterone appears to enhance the PG biosynthetic capacity of the secretory endometrium. This is demonstrated by the in-vitro studies employing cell culture techniques and by maintaining endometrial explants in culture (Smith *et al.* 1984; Kelly *et al.* 1984; Abel & Baird 1980). Conversely, progesterone has been shown to suppress the release of PGs from the endometrium (Abel & Baird 1980; Kelly & Smith 1987). Studies in-vitro have reported a reduction of both the oestradiol-stimulated and the basal PG production by progesterone (Abel & Baird 1980; Kelly & Smith 1987). Furthermore, during pregnancy, when progesterone levels are high, basal endometrial PG production is also reduced (Maathuis *et al.* 1978). It had been suggested that this effect might involve the inhibitory effect of progesterone on the PLA_2 activity (Bonney *et al.*1987).

The withdrawal of progesterone from an endometrium that has been primed with progesterone and oestradiol results in an increase COX-2 expression, while continuing exposure to progesterone is associated with low levels of COX-2 expression (Critchley *et al.* 1999). Evidence for increased PG activity by antagonising progesterone also comes from in-vitro data, which showed a dose-dependent induction of PGF2α release from endometrial stromal cells (Kelly *et al.* 1986) and also from the in vivo observation of increased uterine contractility after mifepristone possibly due to increase PGs (Norman *et al.* 1991; Gemzell-Danielsson *et al.* 1994). This is further supported by the inhibition of glandular PGDH expression seen after administration of antiprogesterones in the early luteal phase and during the early pregnancy (Cheng *et al.* 1993; Cameron *et al.* 1996).

A decrease in the uterine $PGF_{2\alpha}$ release (Gemzell-Danielsson *et al.* 1994), and in the luminal expression of COX-2 had been reported following mifepristone in the early-luteal phase (Marions *et al.* 1999). However, in the early-luteal phase progesterone values are relatively low, and as a consequence the PR and ER expression is maximal, while the converse is true for the mid lueal phase of the cycle. Therefore, in the early luteal phase mifepristone may prevent the effects that are to be exerted by progesterone, where as in the mid-luteal phase it may antagonize the actions of progesterone, which at that time appears to be the suppression of PGs release.

Our results are consistent with previous reports (Cameron *et al.* 1996; Jones *et al.* 1997) that demonstrated localisation of PGDH & COX-2 in the glandular epithelium and, also supports the in-vitro evidence that glands to be the main site for PG synthesis (Lumsden *et al.* 1984; Smith *et al.* 1988).

In our study, there was weak staining for PR in the nuclei of stromal cells in the control samples with minimal ER staining. AR staining was confined to stroma. There was no change in intensity or distribution of staining for steroid receptors after mifepristone (Figures 6.5, 6.6 & 6.7).

Previous studies have reported an increased expression of PR & ER in both glandular and stromal compartments (increase more pronounced in the glands) in the mid-luteal endometrium after early luteal phase administration of antiprogesterones (Berthois *et al.* 1991; Maentausta *et al.* 1993; Cameron *et al.* 1996). In all these studies the endometrium was sampled at least 48 hours or more later than mifepristone. We did not observe a significant change in the endometrial immunoreactivity for all three steroid receptors (ER, PR, and AR) after mifepristone. PR level in the mid-luteal endometrium is minimal compared to the early luteal phase. This may be a reason why we did not see the same level of progesterone antagonism in steroid receptor expression as seen after the early luteal phase administration. Furthermore, the studies mentioned above described effects

seen more than 48 hours after the administration of mifepristone hence; their results may not be comparable to our observations. There are further suggestions that the predominant PR subtype in endometrial glands to be different in early and mid luteal phase, and the functional diversity of these receptor subtypes may explain the different results observed (Mote *et al.* 1999).

Although others have reported barely detectable AR immunoreactivity in the glandular epithelium (Slayden *et al.* 2001), untreated normal mid-luteal endometria in our study exhibit weak glandular and surface epithelial staining. Immediately before menstruation in the late secretory phase, a decrease in stromal AR immuno-staining had been reported. In contrast, an up-regulation was observed in glands as well as in stroma, following the early luteal phase administration of mifepristone (Slayden *et al.* 2001). Once more, here the biopsy was taken more than 48 hours after administering mifepristone.

The distinct endometrial effects seen after the mid-luteal administration of mifepristone add to our understanding of the mechanism of menstruation. There is overwhelming evidence that PGs are involved in the process of normal menstruation (Baird 1996 and references therein). Our results show a down-regulation of PGDH expression and a simultaneous up-regulation of COX-2 expression after administering mifepristone in the mid-luteal phase. Therefore, we conclude that mifepristone induces endometrial-bleeding in the mid-luteal phase by a mechanism involving both PGDH and COX-2 to increase local PG levels in the endometrium.

CHAPTER 7

METHODS

7.1 Hormone assays

7.1.1 Serum progesterone assay

Venous blood was collected, centrifuged to separate serum, and stored serum was later assayed for progesterone using Coat-A-Cont progesterone procedure (solid-phase radioimmunoassay; Diagnostic Products (UK) Ltd., Glyn Rhomwy, Llanbersi, Caernarfon, Gwwyedd, North Wales, UK).

7.1.2 Urinary hormone assays

Urine samples were frozen and later assayed in batches (with all samples from one subject assayed in a single batch) for measurement of urinary LH, E3G and P3G.

Quantitative assessment of urinary LH was performed using a LH MAI Aclone kit (BIOSTAT-DIAGNOSTICS, Stockport, Cheshire, UK). This method incorporates two high affinity monoclonal antibodies into an immuno-radiometric assay system and offers a working range of 1.5 – 200 mIU/ml. Urinary P3G was measured using a direct enzyme immunoassay (sensitive working range 0.16 ug/L – 20 ug/L), while direct immunoassay was used to measure E3G levels (working range 8.36 nmol/L - 2140 nmol/L). Intra-assay coefficients of variation were 6% for E3G, 10% for P3G, and 3% for LH (Yong *et al.* 1992). Geometric means of daily replicates were divided by the respective daily creatinine concentration to correct for variations in the dilution of the urine specimen.

7.2 Statistical Analysis

Both pragmatic and explanatory studies were undertaken as a part of this thesis. For example, studies that evaluated the mechanism of action of LNG and vaginal bleeding (mifepristone induced) belong to the explanatory category while the feasibility of administration of mifepristone, as a once-a-month pill was a pragmatic trial. During the pragmatic trials we tried to mimic real life yet close monitoring and frequent laboratory tests were required during the explanatory studies.

The following statistical tests were used for statistical analysis of the results.

7.2.1 t-test

Employing the t-test assessed a statistically significant difference between two normally distributed sample means (example, Chapter 2).

7.2.2 Confidence intervals

A significance test and a p-value do not explain the precision of the estimated treatment effect. Quoting a confidence interval, or the range of possible differences with which the data were consistent, allowed an assessment of both the statistical and clinical significance (example, Chapter 4).

7.2.3 Kruskal-Wallis ANOVA test

When the normal distribution with uniform variance cannot be assumed in a data set, Kruskal Wallis ANOVA test was used to analyse the variances by ranks (example, Chapter 6).

7.2.4 Correlation

In chapter 4, statistical testing on correlation was done on data aggregated to patient level. Thus, for example, correlating each patient's age with the percentage compliance over all her cycles tested the association between age and compliance. Since both of these variables were quantitative and not very skewed we felt Pearson's correlation was appropriate. Similarly, for binary factors such as whether or not a patient had had previous children, the percent compliance was compared between these two patient groups using a two-sample t-test, which is in fact identical to the Pearson correlation. Clearly chi-squared or Fisher tests are not applicable in this type of analysis where a continuous outcome is being used.

7.2.5 The Dunn's multiple comparisons test

This multiple comparison procedures provided a way to compare and identify the differences in the means of discontinuous data sets with dissimilar numbers in each group (example, Chapter 4).

7.2.6 Mann-Whitney U test

Apparently skewed data were analysed with a non-parametric Mann-Whitney U test (for example, in Chapter 2 the comparison of the difference between urinary E3G levels on the day of LNG treatment in cycles that had delayed LH peak and the 8 cycles that did not).

7.3 Endometrial collection and processing

7.3.1 Endometrial sampling

Both anterior and posterior uterine endometrium was sampled using a Pipelle endometrial sampling device (Prodimed, Neuilly-en-Thelle, France). This disposable, plastic device is 3 mm in diameter and can be easily introduced through the cervical canal into the uterine cavity with minimal discomfort to the un-anaesthetised woman. Once the device has reached the uterine cavity, suction can be created within the device cavity allows the aspiration of a small endometrial sample.

7.3.2 Tissue fixation, processing and microtomy

Tissue samples were cut into small pieces (to aid faster fixation), fixed immediately in 4% paraformaldehyde, for 24 hours at 4^0 C. This fixation preserves the intra-cytoplasmic protein - structure, by forming cross-linking bridges. The tissue biopsy was then stored in 70% ethanol before routinely wax embedding in a Leica TP 1050 (Leica UK Ltd, Milton Keynes, UK). Once the fixation was complete, the tissue was dehydrated (via passage through a ascending grades of alcohol) and was embedded in paraffin wax. 5 μm thick sections were cut from the paraffin blocks using a microtome (HM 325, Shandon, Cheshire, UK), and the sections were dried on glass slides (Superfrost plus, BDH, UK) at 37^0 C overnight.

7.4 Immmunohistochemistry

An avidin Biotin Peroxidase Complex Method (ABC) of antigen detection was utilised for the immuno-staining protocols. This method utilises the high affinity of avidin for biotin. After the addition of an antigen specific primary antibody, a biotinylated secondary antibody (where biotin has been covalently linked to the antibody) was used to link the primary antibody to a subsequently applied complex

of avidin, biotin, and the enzyme horseradish peroxidase (ABC complex). Since avidin has a high affinity and 4 binding sites for biotin, unoccupied sites on the avidin of the ABC complex bind to the biotin of the biotinylated secondary antibody. The reactions were visualised by the addition of the peroxidase substrate, hydrogen peroxide (H_2O_2) and 3, 3' Diaminobenzidine Tetrahydrochloride (DAB). DAB acts as a chromogen and is oxidised during the enzyme-substrate reaction to produce an insoluble dark brown end product.

7.4.1 PR immunostaining protocol

Tissue sections were dewaxed in Histoclear (National Diagnostics, Yorkshire, UK), and rehydrated in descending grades of ethanol in water. Then they were washed in distilled water, and 0.01M phosphate buffered saline (PBS tablets, Sigma, Dorset, UK, pH 7.4 - 7.6) for 10 minutes. Tissue sections were microwaved at full power in 0.01M Sodium Citrate buffer (pH 6.0) for 10 minutes and then allowed to stand in the microwave oven for further 20 minutes, as an antigen retrieval measure. In order to block non-specific endogenous peroxidase activity, following another wash with PBS, the sections were incubated in 3% hydrogen peroxide in distilled water for 10 minutes at room temperature (RT). After a further wash in PBS, a non-immune block was performed, by incubating the tissue sections in normal horse serum (Vector Laboratories, Peterborough, UK) for 20 minutes at RT. The sections were then incubated with a mouse monoclonal anti-human PR antibody at a dilution of 1:40 (0.88 μg/ml protein; Novocastra Laboratories, Newcastle upon Tyne, UK). Mouse IgG at a matching immunoglobulin concentration as the primary antibody (1:6000 dilution) was substituted for the anti-human PR antibody on negative control sections and from this step forward negative control sections were washed separately. Late-proliferative endometrium was included as a positive control tissue, and it is known to express high levels of PR. Following the primary antibody binding, sections were washed in Phosphate Buffered Saline with Tween (PBST). Thereafter the tissue sections were incubated with biotinylated horse anti-mouse IgG (Vectastain, Vector laboratories, Peterborough, UK) at RT for 30 minutes. After a further 10 minutes of washing in PBST, sections were incubated at RT for 30 minutes with a complex of avidin and biotin coupled to a horseradish peroxidase enzyme (Vectastain Elite,

Vector Laboratories, Peterborough, UK). Sections were washed again before the antigen bound ABC-HRP enzyme was identified by applying 3,3'-diaminobenzidin in hydrogen peroxide (DAKO liquid DAB substrate, DAKO Laboratories, High Wycombe, UK). Sections were then washed in water before counterstaining with the non-specific nuclear stain, Harris' Haematoxylin (Pioneer Research Chemicals Ltd, Essex, UK). Finally, sections were washed in scott's tap water, dehydrated by passage through graduated ethanol of increasing concentration, cleared by immersing in Xylene for at least 30 minutes and were mounted in Pertex (Cellpath, Hemel Hampstead, UK).

Similar protocols were employed for oestrogen receptor (ER), androgen receptor (AR), Prostaglandin dehydrogenase (PGDH), and Cyclooxygenase–2 (COX-2). Specific features for each protocol were as follows.

7.3.3 ER immunostaining protocol

Primary monoclonal anti-human ER antibody ER1D5 (0.58 μg/ml protein; DAKO Corp. Laboratories, High Wycombe, UK) was used at dilution of 1:400. Non-immune mouse IgG at the equivalent concentration (1:2400) was used in place of the primary antibody in the negative controls. Sections were incubated with the secondary antibody and ABC complex (Vectastain Elite, Vector Laboratories, Peterborough, UK) for 1 hour. Mid proliferative endometrial sections were included as positive controls.

7.3.4 AR immunostaining protocol

Sections were subjected to a different antigen retrieval step, in which they were pressure cooked (Tefal, Nottingham, UK) in 0.01M Sodium Citrate buffer (pH 6.0) for 5 minutes and then were allowed to rest in buffer for a further 20 minutes. After the wash that followed the endogenous peroxidase block, slides were incubated with Avidin for 15 minutes followed by Biotin for 15 minutes (Avidin-Biotin blocking kit, Vector Laboratories, Peterborough, UK) with a PBS wash in between, to block endogenous Biotin activity. Slides were then incubated overnight at 4^0 C with monoclonal mouse anti-human AR antibody (F 39, BioGenex Laboratories, Inc.

antibody, A Merarini Diagnostics, Berkshire, UK) at a 1:480 dilution in PBS/BSA/Gelatin gel (see recipes, table 7.2). Sections were incubated with the secondary antibody and ABC complex for 1 hour. Mouse IgG at a 1:600 dilution was used for negative control sections, and mid proliferative endometrium was included as a positive control.

7.3.5 PGDH immunostaining protocol

A polyclonal rabbit anti-human PGDH antibody (Dr H. H. Tai, University of Kentuckey, Lexington, USA) was used to identify PGDH immunoreactivity with an avidin-biotin peroxidase detection system. An antigen retrieval step was not required to expose this cytoplasmic epitope. Slides were exposed to a non-immune block by incubating in 20% normal goat serum (SAPU, BSA grade) for 20 minutes. Sections were then incubated with the primary antibody at a 1:3000 dilution overnight at 4^0 C, and rabbit IgG at 1:6000 dilution, substituted the primary antibody for negative controls. Biotinylated goat anti-rabbit was applied for 30 minutes at RT followed by the ABC-complex (Vectastain, Elite ABC Kit, Vector Laboratories, Peterborough, UK) for 60 minutes at RT. Late 3^{rd} trimester fetal membranes was included as a positive control.

7.3.6 COX-2 immunostaining protocol

Detection of COX-2 immunoreactivity employed a polyclonal goat anti-human COX-2 antibody (0.3 μg/ml protein; Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA) with the avidin-biotin peroxidase detection system. Endogenous peroxidase activity was quenched with 3% H_2O_2 in methanol for 30 minutes at RT. An antigen retrieval step was conducted when the sections were pressure cooked in 0.01 M Sodium Citrate buffer (pH 6.0) for 2 minutes and then allowed to rest in the pressure cooker (Tefal, Nottingham, UK) for a further 20 minutes. Incubating in avidin and biotin blocked endogenous biotin, as described for the AR immunostaining protocol. A non-immune block was conducted by incubating the sections in 20% normal rabbit serum (Diagnostics Scotland, UK) for 20 minutes, followed by a 1 hour incubation of the sections with the primary antibody at a 1:600 dilution at 37^0 C. Goat IgG at a 1:3000 dilution (matching IgG concentration as primary antibody) was substituted

for negative control slides. Sections were then incubated in a 1:500 dilution of biotinylated horse anti-goat antibody (Vector Laboratories, Peterborough, UK) for 30 minutes at RT followed by the ABC-complex (DAKO Laboratories, High Wycombe, UK) for 20 minutes at RT. Menstrual endometrium was included as a positive control tissue.

7.4.6 Scoring and immunohistochemistry analysis

We employed a semi-quantitative subjective scoring system to evaluate the intensity and the localization of immunoreactivity in entire tissue sections. Previously we have reported that the immunostaining patterns in endometrial sections measured by the subjective semi-quantitative scoring, showed an almost perfect correlation (regression coefficient of 0.963) with that measured objectively by computerized image analysis (Wang *et al.* 1998). Therefore, the less time consuming, semi-quantitative scoring system provides a valid score suitable for graphical presentation.

Two independent observers using light microscopy visually assessed all coded sections. The two separate scores were then compared to obtain a more objective final score for each section. Once the final score had been agreed for all sections in the five-immunostaining runs, the code was broken, then the final immuno-staining scores were analysed.

The immuno-staining intensity of the steroid receptors (PR, ER, and AR) and COX-2 were scored using a four point scoring scale, where the intensity of staining was assigned as 0 = none, 1 = weak, 2 = distinct, 3 = strong. However, the staining intensity of PGDH showed a narrow range and therefore we adapted a three point scoring scale where the score of zero indicated an absence of immunoreactivity; 1 = faint immunoreactivity; 2 = strong immunoreactivity.

Table 7.1 *Antigens*

Antigen	***Normal Serum***	***Primary antibody***	***Dilution of antibody***	***Secondary antibody***
PR	Horse	Mouse monoclonal anti-human PR antibody (Novocastra Laboratories, Newcastle upon Tyne, UK)	1:40	Biotinylated horse anti-mouse
ER	Horse	Mouse monoclonal anti-human ER antibody ER1D5 (DAKO Laboratories, High Wycombe, UK)	1:400	Biotinylated horse anti-mouse
AR	Horse	Monoclonal mouse anti-human AR antibody (F-39, BioGenex antibody, A Merarini Diagnostics, UK)	1:480	Biotinylated horse anti-mouse
PGDH	Goat	Rabbit polyclonal antibody (Dr H.H.Tai, University of Kentuckey, Lexington, USA)	1:3000	Biotinylated goat anti-rabbit
COX-2	Rabbit	Goat polyclonal anti-human COX-2 antibody (Santa Cruz, Biotechnology, UK)	1:600	Biotinylated horse anti-goat

Table 7.2 *Recipe*

	Recipe	
• Phosphate Buffered Saline (PBS) pH 7.4 – 7.6.	PBS tablets (Sigma, UK) Distilled water	5 tablets 1 litre
• Phosphate Buffered Saline with Tween (PBST) pH 7.4 – 7.6.	PBS as above Sodium chloride Tween 20	1 litre 8 g 100 µl
• PBS/BSA/gel	Sodium dihydrogen orthophosphate 1-hydrate 0.345 g, Sodium chloride 0.405 g, Gelatin 0.05 g, Bovine serum albumin (Fraction V) 0.5 g, 2 g Potassium hydrogen carbonate, and distilled water 50 ml.	
• Scotts tap water	Magnesium sulphate 7-hydrate 20 g, Water 1 litre	

CHAPTER 8

CONCLUSIONS

The main focus of the studies undertaken as a part of this thesis was to evaluate means to advance contraceptive research methodology, while broadening our current understanding of the anti-fertility potentials of two progesterone receptor modulators: antiprogesterone compound mifepristone, and the synthetic progestin LNG.

When LNG was administered twice at a dose of 0.75 mg to 12 healthy female volunteers in the fertile period (immediately before ovulation) of the menstrual cycle, it aborted the LH peak, delayed ovulation and lengthened the cycle in four women (25%). One woman did not ovulate at all despite having an LH surge 2 days after taking LNG. Seven women had apparently normal ovulatory cycles after taking LNG. It is apparent that different women respond differently to the administration of LNG. In all twelve volunteers the luteal phase was significantly shortened following treatment with LNG as compared with the placebo cycles (mean length 11.5 days (SD +/-1.8) vs. 12.9 days (SD +/-2.5) $p = 0.005$). The eight women in whom pre-ovulatory LNG did not affect the cycle length, showed a significant ($p = 0.01$) decrease in total LH in the treatment cycles (18.66 U/mol creatinine (SD +/-8.9)) as compared with the placebo cycles (27.08 U/mol creatinine (SD +/-13.57)). We suggest that LNG taken immediately before ovulation acts as an EC by delaying or preventing ovulation. It may also have an effect on luteal function. Other plausible actions of LNG including retardation of the endometrium, interfering with sperm motility and altering cervical mucus may be important, and need to be explored further.

The limiting factor in the once-a-month approach to administering anti-progesterone as a contraceptive pill is the accurate detection of the LH peak. Clearly, the failure to detect the LH peak accurately has a big impact on the overall effectiveness of the method. The home-use fertility monitor provided us with an opportunity to overcome these problems. In our own control group, if sexual intercourse took place on a fertile day the probability of a pregnancy was 0.25 - 0.32. Thirty two women in the treatment group contributed to a total of 178 cycles and there were two pregnancies (probability of pregnancy 0.01). Imperfect use of the system accounted for failure to identify an LH surge in 11.8% cycles while 7.9% were due to monitor

method failure. During the course of the study we developed an algorithm for the administration of mifepristone if an LH surge was not identified. In nineteen exposure cycles (out of 28 cycles in which an LH surge was not identified) mifepristone was administered using this algorithm and there were no pregnancies. Given that in real life the available methods detecting LH surge are not 100% accurate, such an algorithm will be essential to time the administration of mifepristone when an LH surge was missed. Treatment with mifepristone in the early luteal phase did not disrupt the cycle length but women reported slight vaginal bleeding in 15% of the cycles. Also in our study, mis-timed administration of mifepristone led to predictable effects. Therefore, the combination of a home-use fertility monitor with once a month administration of mifepristone (especially if mifepristone is administered at the early luteal phase) is an acceptable contraceptive option with minimal side effects. Unfortunately, it is difficult to envisage how an easier way of defining the correct timing, which required less compliance, could be devised.

Poor compliance can make the results of a study invalid, yet is commonly overlooked in contraceptive research. We were able to compare the incidence of non-adherence detected by a home use fertility monitor with the traditional self-reported incidence of compliance in a group of women who took part in a trial assessing the efficacy of a novel method of contraception. Women failed to perform 24.6% of the tests in the 162 cycles analysed. In 42% of the cycles they missed tests during a critical period, which could have increased their risk of an unwanted pregnancy. During this critical period, women missed fewer tests and admitted to missing even less. Poor compliance was associated with younger women, those who discontinued the study before completion, and cycles where women were not at risk of pregnancy. The use of microelectronic monitoring systems may improve our understanding of the extent of the problem of patient non-compliance, providing objective information that no other monitoring technique can produce. This understanding provides the opportunity to make the optimum use of potentially effective treatments while validating research evidence.

A reliable and simple means to predict the potentially fertile period can assist in contraceptive research in monitoring frequently changing hormone levels, and in identifying the fertile period. In a significant number of cycles the home use fertility monitor indicated high fertility long before the ovulation had occurred and thus, there is really no advantage to providing this advance warning either in a clinical or a research setting. Conversely, as the rise in LH is the most predictable method of determining the days of high fertility, an error of just around 8% in LH detection can be tolerated. Women missing about 19% of the urine tests requested by the monitor is obviously significant, and the monitor offered us an important methodological advance in providing reliable data on the incidence of non-compliance with urine testing, and exactly when a test was missed. Therefore, this microelectronic system in detecting the urinary LH peak can be invaluable in contraceptive research because it is easy to use and provides reliable data on compliance.

The distinct endometrial effects seen after the mid-luteal administration of mifepristone may add to our understanding of the mechanism of menstruation. There is overwhelming evidence that PGs are involved in the process of normal menstruation. All women in our study reported vaginal bleeding commencing at 24 to 48 hours after taking 200 mg of mifepristone in the mid-luteal phase. In 13 out of the 16 subjects, this was followed by a second bleed at the expected time of the next menses. Although mifepristone significantly depressed the serum progesterone value in all women, the occurrence of a second bleed in the majority (81.25%) suggested only partial luteolysis and a direct effect of mifepristone on the endometrium. Our results show a down-regulation of PGDH expression and a simultaneous up-regulation of COX-2 after administering mifepristone. Therefore, we conclude that mifepristone induces endometrial-bleeding in the mid-luteal phase by a mechanism involving both PGDH and COX-2 to increase local PG levels in the endometrium. However, these preliminary data require to be confirmed by a larger study in the future.

In summary, the studies carried out as part of this thesis have demonstrated the importance of understanding the basic mechanisms of progesterone receptor

modulators in various aspects of contraceptive research. They also confirmed the contraceptive potential of mifepristone as a once-a-month pill, and demonstrated that levonorgestrel acts as an EC mainly through directly or indirectly inhibiting ovulation. Moreover, these studies have established the importance of patient non-compliance while recommending a potential methodological modification to detect the incidence of non-compliance in contraceptive research. Finally, the study that evaluated mifepristone induced bleeding, broadened our current understanding of the mechanism of endometrial bleeding by illustrating the synergistic changes on PG synthetic and catabolic enzymes that may produce an increase in the local endometrial concentration of PGs.

BIBLIOGRAPHY

Abel, M., Baird, D.T. (1980) The effect of 17β-Estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. Endocrinology 106: 1599-1606.

Adlercreutz, H., Brown, J., Collins, W. (1982) The measurements of urinary steroid glucuronides as indices of the fertile period on women. J Steroid Biochem. 17: 695-702.

Affandi, B., Cekan, S.Z., Boonkasemsanti, W., Samil, R.S., Diczfalusy, E. (1987) The interaction between sex hormone binding globulin and levonorgestrel released from Norplant, an implantable contraceptive. Contraception 35: 135-145.

Aitken, R.J., Buckingham, D.W., Irvine, D.S. (1996) The extra-genomic action of progesterone on human spermatazoa: evidence for a ubiquitous response that is rapidly down-regulated. Endocrinology 137: 3999-4009.

Aladin Chandrasekher, Y., Melner, M.H., Nagalla, S.R., Stouffer, R.L. (1994) Progesterone receptor, but not oestradiol receptor, messenger ribonucleic acid is expressed in luteinizing granulosa cells and the corpus luteum in rhesus monkeys. Endocrinology 135: 307-314.

Allan, G.F., Leng, X.H., Tsai, S.Y., Weigel, N.L., Edwards, D.P., Tsai, M.J., O'Malley, B.W. (1992) Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. J Biol Chem 267: 19513-19520.

Allen, W.M., Wintersteiner, O.S. (1934) Crystalline progestin. Science 80: 190-191.

Aono, T., Miyake, A., Kingugusa, T., Kurachi, K. (1976) Progesterone advancement of the oestrogen-induced luteinizing hormone release during the mid-follicular phase in normal cyclic women. J Clin Endocrinol Metab. 71: 451-452.

Akerlund, M. (1991) Function of blood vessels relative to implantation. Baillieres Clin Obstet Gynaecol 5: 15-23

Araki, S., Chikazawa, K., Motoyama, M., Ijima, K., Abe, N., Tamada, T. (1985) Reduction in pituitary desensitization and prolongation of gonadotrophin release by oestrogen during continuous administration of gonadotrophin-releasing hormone in women: Its antagonism by progesterone. J Clin Endocrinol Metab. 60: 590-598.

Back, D.J., Killick, S.R., Stevenson, P.J., Shenoy, N., Elstein, M., Cohen, M. (1987) The relative bioavailability of levonorgestrel and ethinylestradiol when administered in tablet and capsule form. Contraception 36: 321-326.

Backstrom, C.T., McNeilly, A.S., Leask, R.M., and Baird, D.T. (1982) Pulsatile secretion of LH, FSH, prolactine, oestrodiol and progesterone during human menstrual cycle. Clinical endocrinology 17: 29-42.

Bain, D.L., Franden, M.A., McManaman, J.L., Takimoto, G.S., and Horwitz, K.B. (2000) The N-terminal Region of the Human Progesterone A-receptor. J Biol Chem, 275: 7313-7320.

Baird, D. (1965) The fifth freedom. BMJ 2: 1141-1148.

Baird, D.T. (1987) A model for follicular selection and ovulation: Lessons from super-ovulation. J Steroid Biochem 27: 15-23.

Baird, D.T. (1990) The selection of the follicle of the month. In: From ovulation to implantation. Evers, J.H.L., and Heineman, M.J. (eds.) Elsevier Science Publishers; Amsterdam. Pp 3-19.

Baird, D.T., Thong, K.J., Hall, C., Cameron, S.T. (1995) Failure of oestrogen induced luteinizing hormone surge in women treated with mifepristone (RU 486) every day for 30 days. Hum Reprod 10: 2270-2276.

Baird, D.T., Cameron, S.T., Critchley, H.O.D., Drudy, T.A., Howe, A., Jones, R.L., Lea, R.G., Kelly, R.W. (1996) Prostaglandins and menstruation. Euro J Obstet & Gynaecol Reprod Biol 70: 15-17.

Baird, D.T. (2000) Mode of action of medical abortion. JAMA 55: 121-125.

Baldi, E., Krausz, C., luconi, M., Bonaccorsi, L., Maggi, M., Forti, G. (2000) Actions of progesterone on human sperm: A model for non-genomic effects of steroids. J Steroid Biochem Mol Biol. 53: 199-203.

Barrett, J.C., Marshall, J. (1969) The risk of conception on different days of the menstrual cycle.Popul Stud 23: 455-461.

Bartelmez, G.W. (1956) Premenstrual and menstrual ischemia and myth of endometrial arteriovenous anastomoses. Am J Anat., 98: 69-95.

Batista, M.C., Cartledge, T.P., Zellmer, A.W., Nieman, L.K., Merriam, G.R., Loriaux, D.L. (1992a) Evidence for a critical role of progesterone in the regulation of the midcycle gonadotropin surge and ovulation. J Clin Endocrinol Metab 74:565-570.

Batista, M.C., Cartledge, T.P., Zellmer, A.W., Nieman, L.K., Merriam, G.R., Loriaux, D.L. (1992b) Delayed endometrial maturation included by daily administration of the antiprogesterone RU 486: A potential new contraceptive strategy. Am J Obstet Gynecol 167: 60-65.

Baulieu, E.E. (1988) RU 486 (an anti-steroid hormone) receptor structure and heat shock protein mol. Wt 90 000 (hsp 90). Hum Reprod 3: 541-547.

Baulieu, E.E. (1989a) Contragestion and other clinical applications of RU 486, an antiprogesterone at the receptor. Science 245: 1351-7.

Baulieu, E.E., Catelli, M.G. (1989b) Steroid hormone receptors and heat shock protein Mr 90,000 (hsp90): a functional interaction? Pardue, M.L., Feramisco, J.R., Lindquist, S. (eds.) In: Stress-induced proteins. Liss, New York, pp. 203-219.

Baulieu, E.E., Binart, N., Cadepond, F. (1989c) Do receptor associated nuclear proteins explain earliest steps of steroid hormone function? Carlstedt-Duke, J., Eriksson, H., Gustafsson, J.A. (eds.) In: The Steroid / Thyroid hormone receptor family and gene regulation. Birhauser Verlag, Basel, pp 301-318.

Baulieu, E.E. (1993) RU 486- A decade on today and tomorrow. In: Clinical applications of mifepristone RU 486 and other antiprogestins. Donaldson, M.S., Dorflinger, L., Brown, S.S., Benet, L.Z. (eds) Washington, Natl Acad Press, pp71-220.

Beato, M., Chalepakis, G., Schauer, M., Slater E.P. (1989) DNA regulatory elements for steroid hormones. J Steroid Biochem 32: 737-747.

Beato, M., Chavez, S., Truss, M. (1996) Transcriptional regulation by steroid hormones. Steroids 61: 240-251.

Beck, C.A., Weigel, N.L., Edwards, D.P. (1992) Effects of hormone and cellular modulators of protein phosphorylation on transcriptional activity, DNA binding, and phosphorylation of human progesterone receptor. Mol Endocrinol 6: 607-620.

Beck, C.A., Estes, P.A., Bona, B.J., Muro-Cacho, C.A., Nordeen, S.K., Edwards, D.P. (1993a) The steroid antagonist RU 486 exerts different effects on the glucocorticoid and progesterone receptors. Endocrinology 133:728-740.

Beck, C.A., Weigel, N.L., Moyer, M.L., Nordeen, S.K., Edwards, D.P. (1993b) The progesterone antagonist RU 486 acquires agonist activity upon stimulation of cAMP signalling pathway. Proc Natl Acad Sci USA 90: 4441-4445.

Behre, H.M., Kuhlage, J.K., Gassner, C., Sonntag, B., Schem, C., Schneider, H.P.G., Nieschlag, E. (2000) Prediction of ovulation by urinary hormone measurements with the home use Clearplan Fertility monitor: comparison with transvaginal ultrasound scans and serum hormone measurements. Hum Reprod 15: 2478-2482.

Belanger, A., Philibert, D., Teutsch, G. (1981) Regio and stereo-specific synthesis of 11β- substituted 19-norsteroids. Steroids 37: 361-382.

Benhamou, B., Garcia, T., Lerouge, T., Vergezac, A., Gofflo, D., Bigogne, C., Chambon, P., Gronemeyer, H. (1992) A single amino acid that determines the sensitivity of progesterone receptors to RU486. Science 255:206-209.

Bergeron, C., Ferenczy, A., Toft, D.O., Schneider, W., Shyamala, G. (1988) Immunohistochemical studies of progesterone receptors in the human endometrium during the menstrual cycle. Lab Invest. 59: 862-869.

Bergstrom, S., Carlson, C.A. (1968) The prostaglandins: a family of biologically active lipids. Pharmacology Reviews 20: 1-48.

Bertagna, X., Bertagna,C., Luton, J.P., Husson, J.M., Girad, F. (1984) The new steroid analog RU 486 inhibits glucocorticoid action in man. J Clin Endocrinol Metab 59: 25-28.

Bertagna, X., Escourolle, H., Pinquier, J.L., Coste, J., Raux-Demay, M.C., Perles, P., Silvestre, P., Luton, J.P., Strauch, G. (1994) Administration of RU 486 for 8 days in normal volunteers: antiglucocorticoid effects with no evidence of peripheral cortisol deprivation. J Clin Endocrinol Metab 78: 375-380.

Berthois, Y., Salat-Baroux, J., Cornet, D., deBrux, J., Kopp, F., and Martin, P.M. (1991) A multi-parametric analysis of endometrial oestrogen and progesterone receptors after post-ovulatory administration of mifepristone. Fertil & Steril, 55 : 574-554.

Bhakoo, H.S., Katzenellenbogen, B.S. (1977) Progesterone modulation of estrogen-stimulated uterine biosynthetic events and estrogen receptor levels. Mol Cell Endocrinol 8:121-134.

Bhiwandiwalla, P.P., Williams, R.S., Servey, L.J., Jacob, J.E. (2001) Assessment of Clearplan Easy fertility monitor in couples seeking conception assistance. Obstet & Gynecol 97(4 –supplement): S29.

Blackmore, P.F., Fisher, J.F., Spilman, C.H., Bleasdale J.E. (1996) Unusual steroid specificity of the cell surface progesterone receptor on human spermatazoa. Mol Pharmacol 49: 727-739.

Bonnar, J., Flynn, A., Freundl, G., Kirkman, R., Royston, P., Snowden, R. (1999) Personal hormone monitoring for contraception. Brit J Fam Plan 24:128-134.

Bonner, C.J., Carr, B. (2002) Medication compliance problems in general practice: detection and intervention by pharmacists and doctors. Aust J Rural Health 10:33-38.

Bonney, R.C., Qiuilbash, S.T., Franks, S. (1987) Modulation of phospholipase A2 activity in human endometrium and amniotic membranes by steroid hormones. J Steroid Biochem 26: 467-472.

Bonney, R.C., Franks, S. (1987) Endometrial phospholipase A2 enzymes and their regulation by steroid hormones. J Steroid Biochem 27: 4-6.

Bourgeois, S., Mester, J., Baulieu, E.E. (1984) DNA binding properties of glucocorticosteroid receptors bound to the steroid antagonist RU 486. EMBO Journal 3: 751-755.

Bourne, T.H., Hagstrom, H.G., Granberg, S., Josefsson, B., Hahlin, M., Hellberg, P., Collins, W.P. (1996) Ultrasound studies of vascular and morphological changes in the human uterus after a positive self-test for the urinary luteinizing hormone surge. Hum Reprod 11: 369-375.

Branch, C.M., Collins, P.O., Collins, W.P. (1982) Ovulation prediction: Changes in the concentrations of urinary oestrone 3 glucuronide, estradiol-17 beta-glucuronide and estriol-16 alpha glucuronide during conceptional cycles. J. Steroid Biochem. 16: 345-347.

Bray, C., Publicover, S., Barratt, C.R. (1999) Progesterone interaction with sperm plasma membrane, calcium influx and induction of the acrosomal reaction. Reprod Med Rev 7: 81-93.

Brenner, R.M., Slayden, O.D. (1994) Oestrogen action in the endometrium and oviduct of rhesus monkeys during RU486 treatment. Hum Reprod 9 (Suppl 1):82-97.

Brenner, R.M., Slayden, O.D. (2002) Antiprogestin action in the non-human primate reproductive tract. Abstracts presented at 2nd International symposium on progestins,progesterone receptor modulators and progesterone antagonists, Siena, Italy.

Brown, A., Cheng, L., Lin, S., Baird, D.T. (2002) Daily low dose mifepristone has contraceptive potential by suppressing ovulation and menstruation: A double blind randomised control trial of 2 and 5mg per day for 120 days. J Clin Endocrinol Metab 87: 63-70.

Burton, K.A., Hillier, S.G., Habib, F.K., Mason, J.I., Critchley, H.O.D. (2000) Spatial and temporal distribution of the androgen receptor in human endometrium. Journal of Reproduction and Fertility. Abstract. Series 25. Abstract 113.

Butenandt, A., Dannenbaum, H. (1934) Uber androsterone: isolierung eines neuen physiologisch unwirksamen steroiderivatives ans mannernarn, seine verknutsung mit dehydroandrosterone und androsterone bin beitrag vurk constitution des androsterone. Z Physiol Chem 229: 192-208.

Cadepond, F., Ulmann, A., Baulieu, E.E. (1997) RU 486 (Mifepristone): Mechanisms of action and clinical uses. Annu Rev Med 48: 129-156.

Cameron, S.T., Critchley, H.O.D., Buckley, C.H., Chard, T., Baird, D.T. (1996) The effects of post ovulatory administration of onapristone on the development of a secretory endometrium. Hum Reprod 11: 40-49.

Cameron, S.T., Critchley, H.O.D., Buckley, C.H., Kelly, R.W., Baird, D.T. (1997) Effect of two antiprogestins (mifepristone and onapristone) on endometrial factors of potential importance for implantation. Fertil & Steril 67: 1046-1053.

Campbell, S., Cameron, I.T. (1998) The origins and physiology of menstruation. In: Clinical disorders of the endometrium and menstrual cycle. Cameron, I.T., Fraser, I.S., Smith, S.K. (eds.) Oxford University Press, Oxford. Pp. 13-30.

Carson-Jurica, M.A., Schrader, W.T., O'Malley, B.W. (1990) Steroid receptor family: structur and functions. Endocrine Rev 11: 201-220.

Casey, M.L., Hemsell, D.L., Johnston, J.M., MacDonald, P.C. (1980) NAD- dependent 15-hydroxyprostaglandin dehydrogenase activity in human endometrium. Prostaglandins 19: 115-122.

Catalan, R., Castellanos, J.M., Palomino, T., (1989) Correlation between plasma estradiol and estrone-3-glucuronide in urine during the monitoring of ovulation induction therapy. Int. J Fertil. 34: 271-275.

Chang, R.J., & Jaffe, R. B. (1978) Progesterone effects on gonadotrophin release in women pretreated with oestradiol. J Clin Endocrinol Metab 47: 119-125.

Chalbos, D., Galtier, F. (1994) Differential effect of forms A and B of human progesterone receptor on estradiol-dependent transcription. J Biol Chem 269: 23007-23012.

Chauchereau, A., Savouret, J.F., Miligrom, E. (1992) Control of biosynthesis and post transcriptional modification of progesterone receptor. Biol Reprod 46: 174-177.

Cheng, L., Kelly, R.W., Thong, K.J., Hume, R., Baird, D.T. (1993a) The effects of mifepristone (RU 486) on prostaglandin dehydrogenase in decidual and chorionic tissue in early pregnancy. Hum Reprod 8: 705-709.

Cheng, L., Kelly, R.W., Thong, K.J., Hume, R., Baird, D.T. (1993b) The effects of mifepristone (RU 486) on the immuno-histochemical distribution of prostaglandin E and its metabolites in decidual and chorionic tissue in early pregnancy. J Clin Endocrinol Metab 77: 873-877.

Cheng, X., Xiao, B. (1997) Effect of once weekly administration of mifepristone on ovarian function in normal women. Contrception 56: 175-180.

Chrousos, G.P., Laue, L., Nieman, L.K., Kawai, S., Udelsman, R.U., Brandon, D.D., Loriaux, D.L. (1988) Glucocorticoids and glucocorticoid antagonists: lessons from RU 486. Kidney Int Suppl 26:S18-23

Clarke, C.L., Sutherland, R.L. (1990) Progestin regulation of cellular proliferation. Endocrine Reviews 11: 266-301.

Clark, J.H.(1979) Female sex steroid receptor function. Monogr Endocrinol. 14:I-XII.

Clifton, D.K., Steiner, R.A., Resko, J.A., Spies, H.G. (1975) Estrogen-induced gonadotropin release in ovariectomized rhesus monkeys and its advancement by progesterone. Biol Reprod 13: 190-194.

Collins, W.P. (1996) Indicators of potential fertility: scientific principles. In: Natural conception Trough Personal Hormone Monitoring (Bonner, J. ed) New York: The Parthenon Publishing Group, pp 13-33.

Conneely, O.M., Lydon, J.P. (2000a) Progesterone receptors in reproduction: functional impact of the A and B isoforms. Steroids 65: 571-577.

Conneely, O.M., Lydon, J.P., De Mayo, F., O'Malley, B.W. (2000b) Reproductive functions of the Progesterone Receptor. J Soc Gynecol Investig 7: supplement 24-32.

Conneely, O.M., Mulac-Jericevic, B., Lydon, J.P., De Mayo, F.J.R. (2001) Reproductive functions of the progesterone receptor isoforms: lessons from knock-out mice. Mol Cell Endocrinol 179: 97-103.

Corner, G.W. & Allen, W.M. (1929) Physiology of the corpus luteum-II: Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. Am J Physiol 88: 326-329.

Couzinet, B., Le Strat, N., Silvestre, L., Schaison, G. (1990) Late luteal administration of the antiprogesterone RU486 in normal women: effects on the menstrual cycle events and fertility control in a long-term study. Fertil & Steril 54: 1039-1044.

Craft, I., Foss, G.L., Warren, R.J., Fotherby, K. (1975) Effect of norestrel administered intermittently on pituitary ovarian function. Contraception 12: 589-598.

Cramer, J.A., Mattson, R.H., Prevey, M.L. (1990) How often is medication taken as prescribed? A novel assessment technique. JAMA 261: 3273-3277.

Critchley, H.O.D., Baird, D.T. (1988) Antigestogen RU 486 increases LH secretion in the luteal phase of the menstrual cycle. J Endocrinol 18 (suppl.): Abstract 95.

Critchley, H.O.D., Wang, H., Kelly, R.W., Gebbie, A.E., Glasier, A.F. (1998a) Progestin receptor isoforms and prostaglandin dehydrogenase in the endometrium of women using a levonorgestrel-releasing intrauterine system. Hum Reprod 13: 1210-1217.

Critchley, H.O.D., Healy, D.L. (1998b) Effects of estrogen and progesterone on the endometrium. In: Estrogen and progesterone in clinical practice. Fraser, I.S., Jansen, R., Lobo, R., Whitehead, M. (eds.) Churchill Livingston, Edinburgh. pp 145-161.

Critchley, H.O.D., Jones, R.L., Lea, R.G., Drudy, T.A., Kelly, R.W., Williams, A.R.W., Baird, D.T. (1999) Role of Inflammatory mediators in human endometrium during progesterone withdrawal and early pregnancy. J Clin Endocrinol Metab. 84: 240-248.

Critchley, H.O.D., Kelly, R.W., Brenner, R.M., Baird, D.T. (2001) The endocrinology of menstruation – a role for the immune system. Clinical Endocrinology 55: 701-710.

Cromer, B.A., Steinburg, K., Gardner, L., Thornton, D., Shannon, B. (1989) Psychological determinants of compliance in adolescents with iron deficiency. Am J Dis Child 143: 55-58.

Croxatto, H.B., Salvatierra, A.M., Croxatto, H.D., Fuentealba, B. (1993) Effects of continous treatment with low dose mifepristone throughout one menstrual cycle. Hum Reprod 8: 201-207.

Croxatto, H.B., Salvatierra, A.M., Croxatto, H.D., Shailaja, K.M. (1994) Actions of ovarian steroid hormones. Knobil, E., Neill, J. (eds.) In: The physiology of reproduction, Second Edition. Raven Press, Ltd., New York, pp.1011-1058.

Croxatto, H.B., Kovacs, L., Massai, R., Resch, B.A., Fuentealba, B., Salvatierra, A.M., Croxatto, H.D., Zalanyi, S., Viski, S., Krenacs, L. (1998) Effects of long term low dose mifepristone on reproductive function in women. Hum Reprod 13: 793-798.

Croxatto, H.B., Fuentealba, B., Brache, V., Salvatierra, A.M., Alvarez, F., Massai, R., Cochon, L., Faundes, A. (2002) Effects of the Yuzpe regimen, given during the follicular phase, on ovarian function. Contraception 65: 121-128.

Csapo, A.I., Pulkkinen, M. (1978) Indispensibility of the human corpus luteum in the maintenance of early pregnancy. Obstetrics & Gynaecology Survey. 33: 69-81.

Dahlstrom, B., Eckernas, S.-A. (1991) Patient computers enhance compliance with completing questionnaires. In: Patient compliance in Medical practice and clinical trials. Cramer, J.A., Spilker, B., (eds.) Raven Press; New York, pp 233-240.

Daunter, B., Chantler, E.N., Elstein, M. (1976) Scanning electrone microscopy of cervical mucus: normal menstrual cycle and pregnancy. Brit J Obstet Gynaec 83: 738-743.

Deraedt, R., Bonnat, C., Busigny, M., Chatelet, P., Coustey, C., Mouren, M., Philibert, D., Pottier, J., Salmon, J. (1985) Pharmacokinetics of RU 486. Baulieu, E.E., Segal, S.J. (eds.) In: The antiprogestin steroid RU 486 and human fertility control. Planeum Press; New York, pp 103-122

Dierschke, D.J., Bhattacharya, A.N., Atkinson, L.E., Knobil, E. (1970) Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey. Endocrinology 87: 850-853.

Dierschke, D.J., Yamaji, T., Karsch, F.J., Weick, R.F., Weiss, G., Knobil, E. (1973) Blockade by progesterone of estrogen-induced LH and FSH release in the rhesus monkey. Endocrinology 92: 1496-501.

Dixon, G.W., Schlesselman, J.J., Ory, H.W., Blye, R.P. (1980) Ethinyl estradiol and conjugated estrogens as postcoital contraceptives. JAMA 244: 1336-1339.

DeMarzo, A.M., Onate, S.A., Nordeen, S.K., Edwards, D.P. (1992) Effects of the steroid antagonist RU 486 on dimerization of the human progesterone receptor. Biochemistry 31: 10491-10501.

Dixon, G.W., Schlesselman, J.J., Ory, H.W., Blye, R.P. (1980) Ethinyl Estadiol and conjugated estrogens as postcoital contraceptives. JAMA 244: 1336-1339.

Djahanbakhe, O., McNeilly, A.S., Warner, P.M., Swanston, I.A., Baird, D.T. (1984) Changes in plasma concentrations of prolactin, FSH, oestradiol, androstenedione and progesterone around the peri-ovulatory surge of LH in women. Clin Endocrinol 20: 463-472.

Dockery, P., Ismail, R.M.J., Warren, M.A., and Cooke, I.D. (1997) The effects of a single dose of mifepristone (RU 486) on the fine structure of the human endometrium during the early luteal phase. Hum Reprod, 12: 1778-1784.

Doherty, P. (1992) Medical abortion. BMJ 304: 573.

Dominik, R., Trussell, J., Walsh, T. (1999) Failure rates among perfect users and during perfect use: a distinction that matters. Contraception 60: 315-320.

Downie, J., Poyser, N.L., Wunderlich, M. (1974) Levels of prostaglandins in human endometrium during normal menstrual cycle. J Physiol 236: 465-472.

Drouin, J., & Labrie, F. (1981) Interactions between 17β estradiol and progesterone in the control of luteinizing hormone and follicle stimulating hormone release in rat anterior pituitary cells in culture. Endocrinology 108: 52-57.

Duffy, D.M., Wells, T.R., Haluska, G.J., Stouffer, R.L. (1997) The ratio of progesterone receptor isoforms changes in the monkey corpus luteum during the luteal phase of the menstrual cycle. Biol Reprod 57: 693-699.

Dubois, C., Ulmann, A., Baulieu, E.-E. (1988) Contragestion with late luteal administration of RU 486 (Mifepristone). Fertil & Steril. 50: 593-596.

Dunson, D.B., Baird, D.D., Wilcox, A.J., Weinberg, C.R. (1999) Day-specific probabilities of clinical pregnancy based on two studies with imperfect measures of ovulation. Hum Reprod 14:1835-1839.

Durand, M., Cravioto, M.C., Raymond, E.G., Duran-Sanchez, O., Cruz-Hinojosa, M.D.L., Castell-Rodriguez, A., Schiavon, R., Larrea, F. (2001) On the mechanism of action of short-term Levonorgestrel administration in emergency contraception. Contraception 64: 227-234.

Edwards, D.P., Weigel, N.L., Nordeen, S.K., Beck, C.A. (1993) Modulators of cellular protein phosphorylation alter the trans-activation function of human progesterone receptor and the biological activity of progesterone antagonists. Breast Cancer Res Treat 27: 41-56.

Edwards, D.P. (1999) Co-regulatory proteins in nuclear hormone receptor action. Vitamins and Hormones 55: 165-218.

Edwards, D.P., Leonhardt, S.A., Gass-Handel, E. (2000) Novel mechanismsofprogesterone antagonism and progesterone receptor. J Soc Gynecol Investig 7 (suppl): S22-24.

Edwards, D.P., Boonyaratanakornkit, V. (2002) Progesterone receptor activation of transduction signalling pathways through SH3 domain interactions. Abstracts presented at 2nd International symposium on progestins,progesterone receptor modulators and progesterone antagonists, Siena, Italy.

Eisen, S. (1991) Developing more clinically meaningful definitions of medication compliance. In: Patient compliance in Medical practice and clinical trials. Cramer, J.A., Spilker, B., (eds.). New York: Raven Press, pp 225-231.

El-Ashry, D., Onate, S.A., Nordeen, S.K., Edwards, D.P. (1989) Human progesterone receptor complexed with the antagonist RU 486 binds to hormone response elements in a structurally altered form. Mol Endocrinol 3: 1545-1558.

Elashry-Stowers, D., Zva, D.T., Speers, W.C., Edwards, D.P. (1988) Immunohistochemical localisation of progesterone receptor in breast cancer with anti-human monoclonal anti-bodies. Cancer Res 48:6462-6474.

Elger, W., Bartley, J., Schneider, B., Kaufmann, G., Schubert, G., Chwalisz, K. (2000) Endocrine pharmacological characterization of progesterone antagonists and progesterone receptor modulators with respect to PR-agonistic and antagonistic activity. Steroids 65: 713-723.

Emans, S.J., Grace, E., Woods, E., Smith, D.E., Klein, K., Merola, J. (1987) Adolescents' compliance with the use of oral contraceptives. JAMA 257: 3377-3381.

Ensor, C.M., Yang, J.-Y., Okita, R.T., Tai, H.-H. (1990) Cloning and sequence analysis of the cDNA for human placental NAD+-dependent 15-Hydroxyprostaglandin Dehydrogenase. J Biol Chem 265: 14888-14891.

Evans, R.M. (1988) The steroid and thyroid receptor super family. Science 240: 889-895.

Falsetti, C., Baldi, E., Krausz, C., Casano, R., Failli, P., Forti, G. (1993) Decreased responsiveness to progesterone of spermatazoa in oligozoospermic patients. J Androl 14: 17-22.

Farkash, Y., Timberg, R., Orly, J. (1986) Preparation of antiserum to rat cytochrome P-450 cholesterol side chain cleavage, and its use for ultrastructural localization of the immunoreactive enzyme by protein A-gold technique. Endocrinology 118:1353-1365.

Fawell, S.E., Lees, J.A., White, R., Parker, M.G. (1990) Characterization and co-localization of steroid binding and dimerization activities in the mouse oestrogen receptor. Cell 60:953-962.

Fehring, R.J., Gaska, N. (1998) Evaluation of the Lady Free Biotester in determining the fertile period. Contraception 57: 325-328.

Filicori, M., Butler, J.P., Crowley, W.F. Jr. (1984) Neurorndocrine regulation of the corpus luteum in the human. J Clin Invest 73: 1638-1644.

Filicori, M., Santoro, N., Merriam, G.R., and Crowley, W.F. (1986) Characterisation of the physiological pattern of episodic gonadotrophin secretion throughout the human menstrual cycle. J Clin Endocrinol Metab. 62: 1136-1144.

Fink, D.L. (1976) Tailoring the consensual regimen. In: Sackett DL, Haynes RB, eds. Compliance with therapeutic regimens. Baltimore, John Hopkins University Press, pp 110-118.

Finn, C.A. (1986) Implantation, menstruation and inflammation. Biological Reviews of the Cambridge Philosophical Society. 61:313-328.

Fletcher, B.S., Kujubu, D.A., Perrin, D.M., Herschman, H.R. (1992) Structure of the mitogen-inducible TIS10 gene and demonstration that the TIS10-encoded protein is a functional prostaglandin G/H synthase. J Biol Chem 267:4338-4344

Flynn, A., Pulcrano, J., Royston, P., Spieler, J. (1991) An evaluation of the Bioself 110 electronic fertility monitor as a contraceptive aid. Contraception 44: 125-139.

Flynn, C.A., McCarthy, Docker, M., Royston, L.P. (1988) The temporal relationship between vaginal fluid volumes obtained with the Rovumeter vaginal aspirator and the fertile phase of the cycle. Human Reproduction 3: 201-205.

Fortune, J., Vincent, S. (1983) Progesterone inhibits the aromatase activity in rat granulosa cells in vitro. Biol Reprod 28: 1078-1089.

Fotherby, K. (1983) Variability of pharmacokinetic parameters for contraceptive steroids. J Steroid Biochem 19: 817-820.

Fotherby, K. (1990) Pharmacokinetic of gestagens-some problems. Am J Obstet Gynecol 163: 323-328.

Fotherby, K. (1994) Pharmacokinetics and metabolism of progestins in humans. In: Goldzier J.W., Fotherby, K. (eds) Pharmacology of the contraceptive steroids. Raven Press, New York pp. 99-126.

Fotherby, K. (1995) Levonorgestrel. Clinical pharmacokinetics. Clin Pharmacokinet 28: 203-215.

Freedman, L.P. (1992) Anatomy of the steroid receptor Zinc finger region. Endocrin Rev 13: 129-145.

Fung, H.Y., Wong W.L., Wong, F.W., Rogers, M.S. (1994) The study of oestrogen and progesterone receptors in the normal human endometrium during the menstrual cycle by immunohistochemical analysis. Gynecol Obstet Invest. 38: 186-190.

Gaillard, R.C., Riondel, A., Muller, A.F., Herrmann, W., Baulieu, E.E. (1984) RU 486: a steroid with antiglucacosteroid activity that only disinhibit the human pituitary-adrenal system at a specific time of day. Proc Natl Acad Sci USA 81: 3879-3882.

Garcia,J.E., Jones, G.S., Wright, G.L. (1981) Prediction of the time of ovulation. Fertil Steril 36: 308-315.

Garcia,E., Bouchard, P., De Brux, J., Berdah, J., Frydman, R., Schaison, G., Milgrom, E., Perrot-Applanat, M. (1988) Use of immunohistochemistry of progesterone and oestrogen receptors for endometrial dating. J Clin Endocrinol Metab. 67: 80-87.

Garcia T, Benhamou, B, Gofflo, D, Vergezac, A, Philibert, D, Chambon, P, Gronemeyer, H. (1992) Switching agonistic, antagonistic, and mixed transcriptional responses to 11 beta-substitued progestins by mutation of the progesterone receptor. Mol Endocriol. 6: 2071-2078.

Garzo, V.G., Liu, J., Ulmann, A., Baulieu, E.E., Yen, S.S.C. (1988) Effects of an antiprogesterone (RU 486) on the hypothalamic-hypophyseal-ovarian-endometrial axis during the luteal phase of the menstrual cycle. J Clin Endocrinol Metab 66: 508-517.

Gass, E.K., Leonhardt, S.A., Nordeen, S.K., Edwards, D.P. (1998) The antagonist RU 486 and ZK 98299 stimulate progesterone receptor binding to Deoxyribonucleic Acid in vitro and in vivo, but have distinct effects on receptor conformation. Endocrinology 139: 1905-1919.

Gemzell-Danielsson, K., Swahn,M.-L., and Bygdeman,M. (1990) Regulation of non-pregnant human myometrial contractility. Effects of anti-hormones. Contraception 42: 323 –335.

Gemzell-Danielsson, K., Svalander P, Swahn,M.-L., Johannisson E, and Bygdeman,M. (1993) Early luteal phase treatment with RU 486 for fertility regulation Hum Reprod 8: 870-873.

Gemzell-Danielsson, K., Svalander P, Swahn,M.-L., Johannisson E, and Bygdeman,M. (1994a) Effects of a single post ovulatory dose of RU 486 on endometrial maturation in the implantation phase. Hum Reprod 9: 2398-2404.

Gemzell-Danielsson, K, Hamberg M. (1994b) The effect of antiprogestin (RU 486) and prostaglandin biosynthesis inhibitor (Naproxen) on uterine fluid PGF2α concentrations. Hum Reprod. 9: 1626-1630.

Gemzell-Danielsson, K., Swahn, M.-L., Westlund P, Bygdeman,M. (1997) Effect of low daily doses of mifepristone on ovarian function and endometrial development. Hum Reprod 12: 124-131.

Gilbert, J.R., Evans, C.E., Haynes, R.B., Tugwell, P. (1980) Predicting compliance with a regimen of digoxin therapy in family practice. Can Med Assoc J 123: 119-122.

Glasier, A.F., Thong, K.J., Dewar, M., Mackie, M., and Baird, D.T. (1992) Mifepristone (RU 486) compared with high-dose estrogen and progestogen for emergency post-coital contraception. N Engl J Med 327: 1041-1044.

Glasier, A.F., and Baird, D.T. (1997) Emergency Postcoital contraception. N Engl J Med 337: 1058-1064.

Glasier, A.F., Smith, K.B., Cheng, L., Ho, P.C., van der Spuy, Z., and Baird, D.T. (1999) An international study on the acceptability of a once-a-month pill. Hum Reprod, 14: 3018-3022.

Goodman, A., Hodgen, G. (1982) Antifolliculogenic action of progesterone despite hypersecretion of FSH in monkeys. Am J Physiol 243: E387-397.

Goebelsmann, U., Hoffman, D., Chiang, S., Woutersz, T. (1986) The relative bioavailability of levonorgestrel and ethinyl estradiol administered as a low-dose combination oral contraceptive. Contraception 34:341-351.

Graham, J.D., Yeates, C., Balleine, R.L., Harvey, S.S., Miliken, J.S., Bilous, A.S., Clarke, C.L. (1996) Progesterone receptor A and B protein expression in human breast cancer. J Steroid Biochem Mol Biol 56: 93-98.

Graham, J.D., Clarke, C.L. (1997) Physiological action of progesterone in target tissues. Endocr Rev. 18: 502-519.

Green, L.W., Mullen, P.D., Friedman, R.B. (1991) Epidemiological and community approaches to patient compliance. In: Patient compliance in Medical practice and clinical trials. Cramer, J.A., Spilker, B., (eds.). New York: Raven Press, pp 373-386.

Greene, K.E., Kettle, L.M., Yen, S.S.C. (1992) Interruption of endometrial maturation without hormonal changes by an antiprogesterone during the first half of luteal phase of the menstrual cycle: a contraceptive potential. Fertil & Steril 58: 338-342.

Greenland, K.J., Jantke, I., Jennatschke, S., Bracken, K.E., Vinson, C., Gellersen, B. (2000) The human NAD+ dependent 15-hydroxyprostaglandin dehydrogenase gene promoter is controlled by Ets and activating protein-1 transcription factors and progesterone. Endocrinology 141: 581-597.

Gronemeyer, H., Benhamou, B., Berry, M., Bocquel, M.T., Gofflo, D., Garcia, T., Lerouge, T., Metzger, D., Meyer, M.E., Tora, L., et al. (1992) Mechanisms of antihormone action. J Steroid Biochem Mol Biol 41: 217-221.

Grunberg, S.M., Weiss, M.H., Spitz, I.M., Zaretsky, S., Kletzky, O., Groshen, S. (1993) Long-term treatment with the oral anti-progestational agent mifepristone (RU 486). In: Adjuvant Therapy of Cancer VII. Salmon, S.E., (ed.) Lippincott Company; Philadelphia, pp. 55-62.

Guillebaud, J. (1998) Time for emergency contraception with levonorgestrel alone. Lancet 352:416.

Guss, E.K., Leonhardt, S.A., Nordeen, S.K., Edwards, D.P. (1998) The antagonist RU 486 and ZK98299 stimulate progesterone receptor binding todeoxyribonucleic acids in vitro and in vivo, but have distinct effectson receptor conformation. Endocrinology 139:1905-1919.

Hague, S., MacKenzie, I.Z., Bicknell. R., Rees, M.C. (2002) In-vivo angiogenesis and progestogens. Hum Reprod 17:786-793.

Hall, J.E., Bhatta, N., Adams, J.M., Rivier, J.E., Vale, W.W., Crowley, W.F. (1991) Variable tolerance of the developing follicle and corpus luteum to GnRH-releasing hormone antagonist – induced gonadotropin withdrawal in the human. J Clin Endocrinol Metab. 72: 993-1000.

Hamilton, C.J.C.M., Hoogland, H.J. (1989) Longitudinal ultrasonographic study of the ovarian suppressive activity of a low dose triphasic oral contraceptive during correct and incorrect pill intake. Am J Obstet Gynecol 161: 1159-1162.

Hapangama, D.K., Glasier, A.F., Baird, D.T. (2001a) The effects of peri-ovulatory administration of Levonorgestrel on the menstrual cycle. Contraception 63:123-129.

Hapangama, D.K., Brown, A., Glasier, A.F., Baird, D.T. (2001b) Feasibility of administering mifepristone as a once a month pill. Hum Reprod 16: 1145-1150.

Hapangama, D.K., Glasier, A.F., Baird, D.T. (2001c) Non-compliance in a group of women using a novel method of contraception. Fertil & Steril 76: 1196-1201.

Hapangama, D.K., Critchley, H.O.D., Henderson, T., Baird, D.T. (2002) Mifepristone Induced Vaginal Bleeding Is Associated With Increased Immunostaining For Cyclooxygenase 2 and Decrease In Prostaglandin Dehydrogenase In Luteal Phase Endometrium. J Clin Endocrinol Metab. 87: 5229-5234.

Hartmann, M., Wettstein, A. (1934) Zur kenntnis der corpus-luteum hormone. Helv Chim Acta 17:1365-1372.

Haynes, R.B. (1979) Introduction. In: Haynes R.B., Taylor, D.W., Sackett, D.L. (eds.) Compliance in Health Care. Baltimore, John Hopkins University Press, pp1-7.

Haynes, R.B. (1986) A critical review of the "determinants of patient compliance with therapeutic regimens. In: Sackett, D.L., Haynes, R.B. (Eds.) Compliance with therapeutic regimens. John Hopkins University Press, Baltimore pp 27-39.

Healy, D.L., Chrousos, G.P., Schulte, H.M., Williams, R.F., Coll, P.W., Baulieu, E.E., Hodgen, G.D. (1983) Pituitary and adrenal responses to the antiprogesterone and antiglucacorticoid steroid RU 486 in primates. J Clin Endocrinol Metab 57: 863-865.

Healy, D.L., Chrousos, G.P., Schulte, H.M., Gold, P.W., Hodgen, G.D. (1985) Increased adrenocorticotropin, cortisol, and arginine vasopressin secretion in primates after the antiglucocorticoid steroid RU 486: dose response relationship. J Clin Endocrinol Metab 60: 1-4.

He, C.H., Shi, Y.E., Liao, D.L., Zhu, Y.H., Xu, J.Q., Matlin, S.A., Vince, P.M., Fotherby, K., Van Look, P.F. (1990) Pharmacokinetic study of two types of post coital contraceptive tablets containing Levonorgestrel. Contraception 41:557-567.

Heikinheimo, O., Haukkamaa, M., and Lahteenmaki, P. (1987) Distribution of RU 486 and its demethylated metabolites in humans. J Clin Endocrinol Metab 68: 270-275.

Heikinheimo, O. (1989) Pharmacokinetics of the antiprogestin RU 486 in women during multiple dose administration. J Steroid Biochem 32:21-25.

Heikinheimo, O., Ratna, S., Grunberg, S., Spitz, I.M. (2000) Alterations in sex steroids and gonadotrophins in post-menopausal women subsequent to long-term mifepristone administration. Steroids 65: 831-836.

Henrion, R. (1989) RU 486 abortions. Nature 338:110.

Herrman, W., Wyss, R., Riondel, A. (1982) (Translation) The effects of an antiprogesterone steroid on women: interruption of the menstrual cycle and of early pregnancy. C.R. Acad.Sci. Paris, t.303, Serie III, 294:933-938.

Hill, N.C., Selinger, M., Ferguson, J., MacKenzie, I.Z. (1991) Trans-placental passage of mifepristone and its influence on maternal and fetal steroid concentrations in the second trimester of pregnancy. Hum Reprod 6: 458-462.

Hirsch, K.E., Goldzieher, J.W., Gibbons, W.E., Besch, P.K. (1986) Evaluation of the OvuStick urinary Luteinizing hormone kit in normal and stimulated menstrual cycles. Obstet Gynecol 67:450-453.

Hoff, J.D., Quigley, M.E., Yen, S.S.C. (1983) Hormonal dynamics at mid cycle: a re-evaluation. J Clin Endocrinol Metab 57: 792-806.

Ho, P.C., Kwan, M.S.W. (1993) A prospective randomized comparison of levonorgestrel with the Yuzpe regimen in post-coital contraception. Hum Reprod 8: 389-392.

Hsueh, A.J.W., Peck, E.J.Jr., Clark, J.H. (1975) Progesterone antagonism of the estrogen receptor and estrogen induced uterine growth. Nature 254: 337-339.

Humpel, M., Wendt, H., Dogs, G., Schulze, P.E., Weiss, C., Speck, U. (1977) Pharmacokinetic parameters of norgestrel, lynestrenol and cyproterone acetate. Contraception. 16:199-215.

Hurley, F.L. (1991) Statistical approach to subgroup analyses: Patient compliance data and clinical outcome. In: Patient compliance in Medical practice and clinical trials. Cramer, J.A., Spilker, B., (eds.). New York: Raven Press, pp 243-250.

Hutchison, J.S., Zeleznik, A.J. (1984) The rhesus monkey corpus luteum is dependent on pituitary gonadotropin secretion throughout the luteal phase of the menstrual cycle. Endocrinology. 115: 1780-1786.

Ismail, M., Arshat, H., Pulcrano, J., Royston, R., Spieler, J. (1989) An evaluation of the Bioself 110 fertility indicator. Contraception 39:53-71.

Jackson, T.A., Richer, J.K., David, L., Bain, D.L., Takimoto, G.S., Tung, L., Horwitz, K.B. (1997) The partial-agonist activity of antagonist occupied steroid receptors is controlled by a novel hinge domain binding co-activator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 11: 693-705.

Janne, O., Kontula, K., Luukkainen T., Vihko, R. (1975) Oestrogen induced progesterone receptor in the human uterus. J Steroid Biochem. 6:501-509.

Johannisson, E.D.B. (1971)Depression of the progesterone levels in women treated with synthetic gestagens after ovulation. Acta Endocrinologica. 68: 779-792.

Johannisson, E., Oberholzer, M., Swahn, M.-L., Bygdeman, M. (1988) Vascular changes in the human endometrium following the administration of the progesterone antagonist RU 486. Contraception 39: 103-117.

Jones, R.L., Kelly, R.W., Critchley, H.O.D. (1997) Chemokines and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation. Hum Reprod 12: 1300-1306.

Jost, A. (1986) (Translation) Animal Reproduction-new data on the hormonal requirement of the pregnant rabbit; partial pregnancies and fetal anomalies resulting from treatment with a hormonal antagonist given at a sub-abortive dosage. C.R. Acad.Sci. Paris, t.303, Serie III, no 7: 281-284.

Juchem, M., Pollon, K.(1990) Binding of progestagens to serum proteins and cytoplasmic receptor. Am J Obstet Gynecol 163:2171-2183.

Kasl, S., Cobb, S. (1966) Health Behaviour, illness behaviour, and sick role behaviour: part I. Arch Environ Health 12: 246-266.

Kasper, K.C., Rodrick-Highberg, G., Lankford, J.C. (1984) OvuStick urine hLH test: an aid for management of fertility in women. Clin Chem 30: 1050-1054.

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P. (1990) Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J 9:1603-1614.

Katzenellenbogen, B.S. (1980) Dynamics of steroid receptor action. Annu Rev Physiol. 42: 17-35.

Khan, F.S., Fotherby, K. (1983) In vitro hydroxylation of norgestrel. J Steroid Biochem 19:1169-1172.

Kelly, R.W., Healy, D.L., Cameron, M.J., Cameron, I.T., Baird, D.T. (1986) The stimulation of prostaglandin production by two antiprogesterone steroids in human endometrial cells. J Clin Endocrinol Metab 62: 1116-1123.

Kelly, R.W., Smith, S.K. (1987) Progesterone and antiprogestins, a comparison of their effect on prostaglandin production by human secretory phase endometrium and decidua. Prostaglandins Leukot Med 29: 181-186.

Kelly, R.W. (1994) Pregnancy maintenance and parturition: the role of prostaglandin in manipulating the immune and inflammatory response. Endocrine Reviews 15: 684-706.

Kerin, J.F., Edmonds, D.K., Warnes, G.M., Cox, L.W., Seamark, R.F., Matthews, C.D., Young, G.B., Baird, D.T. (1981) Morphological and functional relations of Graafian follicle growth to ovulation in women using ultrasonic, laparoscopic and biochemical measurements. Br J Obstet Gynaecol 88:81-90.

Kesseru, E., Garmendia, F., Westphal, N., Parada, J. (1974) The hormonal and peripheral effects of d-norgestrel in postcoital contraception. Contraception 10: 411-424.

Kirkland, J.L., Murthy, L., Stancel, G.M. (1992) Progesterone inhibits the estrogen-induced expression of c-fos messenger ribonucleic acid in the uterus. Endocrinology 130:3223-3230

Khan, F.S., Fotherby, K. (1983) In vitro hydroxylation of norgestrel. J Steroid Biochem 19:1169-1172.

Klopper, A. (1973) Endocrinological effects of oral contraceptives. Clinics in Endocrinology and Metabolism 2:489-502.

Knobil, E. (1980) The neuro-endocrine control of the menstrual cycle. Recent progress in Hormone Research 36: 53-88.

Kook, K., Gabelnick, H., Duncan, G. (2002) Pharmacokinetics of levonorgestrel 0.75 mg tablets. Contraception 66:73.

Kubba, A.A., White, J.O., Guillebaud, J., Elder, M.G. (1986) The biochemistry of human endometrium after two regimens of postcoital contraception: a dl-norgestrel/ethinylestradiol combination or danazol. Fertil & Steril 45:512-516.

Kuhnz, W., Al-Yacoub, G., Furhrmeister, A. (1992) Pharmacokinetics of LNG after a dose of 150μg. Contraception 46: 443-454.

Kumar, V., Green, S., Stack, G., Berry, M., Jin, J.R., Chambon, P. (1987) Functional domains of the human estrogen receptor. Cell 51:941-951.

Kuntz, G., Bell, D., Deininger, H., Wildt, L., Leyendecker, G. (1996) The dynamics of rapid sperm transport through the female genital tract: Evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. Hum Reprod 11: 627-632.

Lahteenmaki, P., Heikinheimo, O., Croxatto, H., Spitz, I., Shoupe, D., Birgerson, L., Luukkainen, T. (1987) Pharmacokinetics and metabolism of RU 486. J Steroid Biochem 27: 859-863.

Lamberts, S.W., Koper, J.W., de Jong, F.H. (1991) The endocrine effects of long term treatment with mifepristone. J Clin Endocrinol Metab 73: 189-191.

Landgren, B.M., Johannisson, E., Aedo, A.R., Kumar, A., Yong-en, S. (1989) The effects of LNG administered in large doses at different stages of the cycle on ovarian function and endometrial morphology. Contraception. 39: 275-289.

Landgren, B.M., Dada, O., Aedo, A.R., Johannisson, E., Diczfalusy, E. (1990) Pituitary, ovarian and endometrial effects of 300μg LNG administered on cycle days 7 to 10. Contraception 41: 569-581.

Lanz, R.B., Rusconi, S. (1994) A conserved carboxy-terminal subdomain is important for ligand interpretation and transactivation by nuclear receptors. Endocrinology 135: 2183-2195.

Laue, L., Lotze, M.T., Chrousos, G.P., Barnes, K., Loriaux, D.L., Fleisher, T.A. (1990) Effect of chronic treatment with the glucocorticoid antagonist RU 486 in man: toxicity, immunological, and hormonal aspects. J Clin Endocrinol Metab 71:1474-80

Laumas, K.R., Farooq, A. (1966) The uptake in vivo of [1,2-3H]-progesterone by the brain and genital tract of the rat. J Endocrinol 36:95-96.

Lebeau, M.C., Binart, N., Cadepond, F., Baulieu, E.E. (1993) Steroid receptor associated proteins: heat shock protein 90 and p59 immunophilin. Moudgil, V.K. (ed.) In: Steroid hormone Receptors: Basic and clinical aspects. Birkhauser, Boston, pp. 261-280.

Ledger, W.L., Sweeting, V.M., Hillier, H., (1992) Inhibition of ovulation by low dose mifepristone (RU 486). Human Reproduction 7: 945-950.

Leonhardt, S.A., Edwards, D.P. (2002) Mechanism of action of progesterone antagonists. Exp Biol Med 227: 969-980.

Lessey, B.A., Killam A.P., Metzger, D.A., Haney, A.F., Greene, G.L., McCarty, Jr K.S. (1988) Immunohistochemical analysis of human uterine oestrogen, and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab. 67: 334-340.

Leyendeker, G., Wardlaw, S., Nocke, W. (1972) Experimental studies on the endocrine regulations during the peri-ovulatory phase of the human menstrual cycle. Acta Endocrinologica 71: 160-178.

Leyendecker, G. (1979) The patho-physiology of hypothalamic ovarian failure. Diagnostic and therapeutical considerations. Eur J Obstet Gynecol Reprod Biol 9:175-86

Leyendecker, G., Waibel-Treber, S., and Wildt, L. (1990) The central control of follicular maturation and ovulation in the human. Oxford Reviews of Reproductive Biology 12: 93- 146.

Leyendecker, G., Kunz, G., Wildt, L., Beil, D., Deininger, H. (1996) Uterine hyperperistalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility. Hum Reprod. 11: 1542-1551.

Lieberman, S., Erlanger, B.F., Beiser, S.M. (1959) Steroid protein conjugates: Their chemical, immunochemical, and endocrinological properties. In: Recent Progress in Hormone Research Vol XV Pincus, G. (ed.) Academic Press, New York and London.

Lieberman, S., Lin, Y.Y. (2001) Reflections on Sterol side chain cleavage process catalyzed by cytochrome P 450 (scc). J Steroid Biochem Mol Biol 78: 1-14.

Li, T.C., Dockery, P., Thomas, P., (1988) The effects of progesterone receptor blockade in the luteal phase of normal fertile women. Fertil & Steril 50: 732-240.

Lim, B.H., Lees, D.A.R., Bjornsson, S., Lunan, C.B., Cohn, M.R., Stewart, P., Davey, A. (1990) Normal development after exposure to mifepristone in early pregnancy. Lancet 257-258.

Ling, W.Y., Wrixon, W., Acorn, T., Wilson, E., Collins, J. (1983a) Mode of action of dl-norgestrel and ethinylestradiol combination in post-coital contraception. III. Effect of pre-ovulatory administration following the luteinizing hormone surge on ovarian steroidogenesis. Fertil & Steril 40:631-636.

Ling, W.Y., Wrixon, W., Zayid, I., Acorn, T., Popat, R., Wilson, E. (1983b) Mode of action of dl-norgestrel and ethinylestradiol combination in postcoital contraception. II. Effect of postovulatory administration on ovarian function and endometrium. Fertil & Steril 39:292-297.

Lippman, M., Monaco, M.E., Bolan, G. (1977) Effects of estrone, estradiol and estriol on hormone responsive breast cancer in long-term tissue culture. Cancer Res, 37: 1901-1907.

Liu, J.H., Garzo, G., Morris, S., Stuenkel, C., Ulmann, A., Yen, S.S. (1987) Disruption of follicular maturation and delay of ovulation after administration of the antiprogesterone RU486. J Clin Endocrinol Metab 65:1135-1140.

Lockwood, C.J., Krikun, G., Hausknecht, V.A., Papp, C., Schatz, F. (1998) Matrix metalloproteinase inhibitor expression in endometrial stroma cells during progestin-initiated decidualisation and menstruation related progestin withdrawal. Endocrinology 139: 4607-4613.

Lui, J.H., & Yen, S.S.C. (1983) Induction of mid-cycle gonadotrophin surge by ovarian steroids in women: a critical evaluation. J Clin Endocrinol Metab. 57: 797-802.

Lumsden, M.A., Kelly, R.W., Baird, D.T. (1983) Primary dysmenorrhoea: the importance of both prostaglandins E2 and F2α. Br J Obstet Gynaecol 90: 1135-1140.

Lumsden, M.A., Brown, A., Baird, D.T. (1984) Prostaglandin production from homogenates of separated glandular epithelium and stroma from human endometrium. Prostaglandins 28: 485-496.

Lusher, T.F., Vetter, H., Siegenthaler, W., Vetter, W. (1985) Compliance in hypertension: facts and concepts. J Hypertens. 3(suppl 1): 3-9.

Luukkainen, T., Heikinheimo, O., Haukkamaa, M., Lahteenmaki, P. (1988) Inhibition of folliculogenesis and ovulation by the antiprogesterone RU 486. Fertil Steril 49: 961-963.

Lydon, J.P., DeMayo, F.J., Funk, C.R., Mani, S.K., Hughes, A.R., Montgomery Jr, C.A., Shymala, G., Conneely, O.M., O'Malley, B.W. (1995) Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev 9: 2266-2278.

Lyons, E.A., Taylor, P.J., Zheng, X.H., Ballard, G., Levi, C.S., Kredentser, J.V. (1991) Characterization of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. Fertil & Steril 55:771-774.

Maathuis, J.B., Kelly, R.W. (1978) Concentrations of prostaglandins F2 alpha and E2 in the endometrium throughout the human menstrual cycle, after the administration of clomiphene or an oestrogen-progesten pill and in the early pregnancy. J Endocrinol 77: 361-371.

Machelon, V., Nome, F., Grosse, B., Lieberherr, M. (1996) Progesterone triggers rapid transmembrane calcium influx and/ or calcium mobilization from endoplasmic reticulum, via a pertussis-insensitive G-protein in granulosa cells in relation to luteinization process. J Cell Biochem 61: 619-28.

Maentausta, O., Svalander, P., Gemzell-Danielsson, K., Bygdeman, M., Vihko, R. (1993) The effects of an antiprogestin , mifepristone, and an antiestrogen, Tamoxifen, on endometrial 17β-hydroxysteroid dehydrogenase and progestin and estrogen receptor during the lutealphase of the menstrual cycle; an immunohistochemical study. J Clin Endocrinol Metab. 77: 913-918.

Mahajan, D.K., London, S.N. (1997) Mifepristone (RU 486): a review. Fertil Steril 6: 967-975.

Mahesh, V.B., & Muldoon, T.G. (1987) Integration of the effects of oestradiol and progesterone in the modulation of gonadotrophin secretion. J Steroid Biochem. 27: 665-675.

Mandelin, E., Koistinen, H., Koistinen, R., Affandi, B., Seppala, M.(1997) LNG releasing intrauterine device wearing women express contraceptive glycodelin-A in endometrium during mid-cycle: another contraceptive mechanism? Hum Reprod 12: 2671-2675.

Mangal, R.K., Wiehle, R.D., Poindexter III, A.N., Hilsenrath, R.E., Weigel, N.L. (1997) Differential expression of uterine progesterone receptor A and B forms during the menstrual cycle. J Steroid Biochem Mol Biol 63: 195-202.

Mani, S.K., Blaustein, J.D., Allen, J.M., Law, S.W., O'Malley, B.W., Clark, J.H. (1994a) Inhibition of rat sexual behaviour by antisense oligonucleotides to the progesterone receptor. Endocrinology 135:1409-1414.

Mani, S.K., Allen, J.M., Clark, J.H., Blaustein, J.D., O'Malley, B.W. (1994b) Convergent pathways for steroid hormone- and neurotransmitter- induced rat sexual behaviour. Science 245: 1246-1249.

March, C.M., Goebelsmann, U., Nakamura, R.M., Mishell, D.R.Jr. (1979) Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle stimulating hormone surges. J Clin Endocrinol Metab 49:507-513.

March, C.M., Marrs, R.P., Goebelsmann, U., Mishell, D.R.Jr. (1981) Feed-back effects of estradiol and progesterone upon gonadotropin and prolactin release. Obstet Gynecol 58:10-16.

Marinker, M. (1997) Personal paper: Writing prescriptions is easy. BMJ 314: 747-748.

Marions, L., Danielsson, K.G. (1999) Expression of cyclo-oxygenase in human endometrium during the implantation period. Mol Hum Reprod 5: 961-965.

Marions, L., Hultenby, K., Lindell, I., Sun, X., Stabi, B., Gemzelle-Danielsson, K. (2002) Emergency contraception with mifepristone and Levonorgestrel: Mechanism of action. Obstetrics & Gynecology 100: 65-71.

Markee, J.E. (1940) Menstruation in intraocular endometrial transplants in the rhesus monkey. Contrib. Embryol. 28: 219-308.

Marsh, M.M. Findlay, J.K., Salamonsen, L.A. (1996) Endothelins and menstruation. Hum Reprod 11: 83-89.

McCarty, K.S. Jr, Miller, L.S., Cox, E.B., Konradt, J., McCarty, K.S. Sr. (1985) Oestrogen receptor analyses: Correlation of biochemical and immuno-histochemical methods using monoclonal anti-receptor anti-bodies. Arch. Pathol. Lab. Med. 109: 716-721.

McDonnell, D.P., Vegeto, E., O'Malley, B.W. (1992) Identification of a negative regulatory function for steroid receptor. Proc Natl Acad Sci USA 89: 10563-10567.

McDonnell, D.P., Shahbaz, M.M., Vegeto, E., Goldman, M.E. (1994a) The human progesterone receptor A form functions as a transcriptional modulator of mineralocorticoid receptor transcriptional activity. J Steroid Biochem Mol Biol 48: 425-432.

McDonnell, D.P., Goldman, M.E. (1994b) RU 486 exerts antioestrogenic activities through a novel progesterone receptor A form mediated mechanism. J Biol Chem 269: 11945-11949.

Meichenbaum D., Turk, D.C. (1987) Facilitating treatment adherence: a practitioner's guidebook. New York: Plenum Press.

Meyer, M.C., Pornon, A., Ji, J. (1990) Agonist and antagonist activities of RU 486 on the functions of the human progesterone receptor. EMBO J. 9: 3923-3932.

Miligrom, E., Baulieu, E.E. (1970) Progesterone in uterus and plasma I. Binding in rat uterus 105,000 supernatant. Endocrinology 87:276-286.

Milne SA, Perchick GB, Boddy SC, Jabbour HN. (2001) Expression, localisation, and signalling of PGE2 and EP2/EP4 receptors in Human nonpregnant endometrium across the menstrual cycle. J of Clin Endocrinol Metab 86: 4453-4459.

Moghissi, K.S., Syner, F.N., McBride, L.C. (1973) Contraceptive mechanism of microdose norethindrone. Obstet Gynec 41: 585-590.

Molloy, B.G., Coulson, K.A., Lee, J.M., Watters, J.K. (1985) "Missed pill" conception: fact or fiction? BMJ 290: 1474-1475.

Mote, P.A., Balleine, R.L., McGowan, E.M., Clarke, C.L. (1999) Co-localisation of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. J Clin Endocrinol Metab. 84: 2963-2971.

Moutsatsou, P., Sekeris, C.E. (1997) Estrogen and progesterone receptors in the endometrium. Ann NY Acad Sci. 816: 99-115.

Mullen, P.D. (1997) Editorial: Compliance becomes concordence. BMJ 314:691.

Natraj, V., Richards, J.S. (1993) Hormone regulation, localization, and functional activity of the progesterone receptor in granulosa cells of rat preovulatory follicles. Endocrinology 133: 761-769.

Nieman, L.K. (1993) The use of antiprogestins in the reproductive cycle. In: Clinical applications of mifepristone RU 486 and other antiprogestins. Donaldson, M.S., Dorflinger, L., Brown, S.S., Benet, L.Z. (eds.) Washington, Natl Acad Press, pp 139-147.

Noe, M., Kunz, G., Herbertz, M., Mall, G., Leyendecker, G. (1999) The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial-subendometrial unit. Hum Reprod 14: 190-197.

Nordeen, S.K., Bona, B.J., Beck, C.A., Edwards, D.P., Borror, K.C., DeFranco, D.B. (1995)The two faces of a steroid antagonist: When an antagonist isn't. Steroids 60: 97-104.

Norman, J.E., Kelly, R.W., Baird, D.T. (1991a) Uterine activity and decidual prostaglandin production in women in early pregnancy in response to mifepristone with or without indomethacin in vivo. Hum Reprod 6: 740-744.

Norman, J.E., Wen Xuan, W., Kelly, R.W., Glasier, A.F., McNeilly, A.S., Baird, D.T. (1991b) Effects of mifepristone in vivo decidual prostaglandin synthesis and metabolism. Contraception 44: 89-98.

Odeblad, E. (1972) Biophysical techniques of assessing cervical mucus and microstructure of cervical epithelium. Cervical Mucus and Human Reproduction (Eds: lstein M, Moghissi KS and Broth R) Scriptor, Copenhagen, p58-74.

Odell, W.D., and Swerloff, R.S. (1968) Progesterone- induced LH and FSH surge in post- menopausal women: a stimulated ovulatory peak. Proceedings of national Academy of Sciences, USA. 61: 529-536.

Oehninger, S., Blackmore, P.F., Morshedi, M., Sueldo, C., Acosta, A.A., Alexander, N.J. (1994) Defective calcium influx and acrosome reaction (spontaneous and progesterone induced) in spermatazoa of infertile men with severe teratozoospermia. Fertil Steril 61: 349-354.

O'Malley, B.W., McGuire, W.L., Kohler, P.O., Korenman, S.G. (1969) Studies on the mechanism of steroid hormone regulation of synthesis of specific proteins. Recent Prog Horm Res 25: 105-151.

Pakarinen, P., Luukkainen, T., Laine, H., Lahteenmaki, P. (1995) The effect of local intrauterine LNG administration on endometrial thickness and uterine blood circulation. Hum Reprod 10: 2390-2394.

Park-Starge, O.-K., Mayo, K.E. (1994) Transient expression of progesterone receptor messenger RNA in ovarian granulosa cells after the preovulatory luteinizing hormone surge. Mol Endocrinol 5: 967-978.

Peck, C.L., King, N.J. (1982) Increasing patient compliance with prescription. JAMA 248: 2874-2877.

Permezel, J.M., Lenton, E.A., Roberts, I, Cooke, I.D. (1988) Acute effects of Progesterone and the antiprogestin RU486 on Gonadotropin Secretion in the follicular phase of the menstrual cycle. J Clin Endocrinol Metab. 68: 960-965.

Peek, M., Landgren, B. -M., Johannisson, E. (1992) The endometrial capillaries during the normal menstrual cycle: a mophometric study. Hum Reprod. 7: 906-911.

Peek, M.J., Markham, R., Fraser, I.S. (1995) The effects of natural and synthetic sex steroids on human endometrial cell proliferation. Hum Reprod 10: 2238-2243.

Peterson, C.M. (2000) Estrogen and progesterone receptors: An overview from the year 2000. J Soc Gynecol Investig 7: supplement 3-6.

Philibert, D., Deraedt, R., Teutsch, G. (1981) RU 486 a potent antiglucocorticoid in vivo. Program of the VIII Int Cong of Pharmacology, Tokyo, Japan. Abstract No: 1463.

Philibert, D. (1984) RU 38486: an original multifaceted antihormone in vivo. In: Adrenal Steroid Antagonism. Agarwal, M.K., ed. Berlin: Walter de Gruyer and Co, pp77 – 101.

Piaggio, G., Von Hertzen, H., Grimes, D.A., Van Look, P.F.A. (1999) Timing of emergency contraception with levonorgestrel or the Yuzpe regimen. Lancet 353: 721.

Pincus, G. (1965) The control of fertility. Academic Press, New York, pp 128-138.

Plant, A., McLaughlin, E.A., Ford, W.C.L. (1994) Intracellular calcium measurements in individual human sperm demonstrate that the majority can respond to progesterone. Fertil Steril 64: 1213-1215.

Potter, L.S. (1991) Oral contraceptive compliance and its role in the effectiveness of the method. In: Cramer JA, Spilker B, eds. Patient compliance in Medical practice and clinical trials. New York: Raven Press, pp195-231.

Potter, L., Okley, D., de Leon-Wong, E., Caneonar, R. (1996) Measuring compliance among oral contraceptive users. Fam Plann Perspect 28:154-158.

Poyser, N.L. (1992) Prostaglandins in animal reproduction. Ag Biotech News and Information 4: 17-25.

Poyser, N.L. (1995) The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukot Essent Fatty Acids 53: 147-195.

Press, M.F., Green, G.L. (1988) Immuno-histochemical localization of oestrogen and progesterone receptors. In: Advances in immuno-histochemistry, Delellis, R.A. (ed.), Raven Press, New York, pp 341-361.

Press, M.F., Udove, J.A., Greene, J.L. (1988) Progesterone receptor distribution in the human endometrium. Analysis using monoclonal antibodies to the human progesterone receptor. Am J Pathol. 131: 112-124.

Probstfiels, J.L., Russell, M.L., Insull, W., Yusuf, S. (1990) Dropouts from a clinical trial, their recovery and characterization: a basis for dropout management and prevention. In: Shumaker SA, Schron EB, Ockene JK, eds. The handbook of health behaviour change. New York: Springer Publishing Co., pp 376-400.

Puller, T., Birtwell, A.J., Wiles, P.G., Hay, A., Feely, M.P. (1988) Use of a pharmacologic indicator to compare compliance with tablets prescribed to be taken once, twice, or three times daily. Clin Pharmacol Ther 44: 540-544.

Queenan, J.T., O'Brien, G.D., Bains, L.M., Simpson, J., Collins, W.P., Campbell, S. (1980) Ultrasound scanning of ovaries to detect ovulation in women. Fertil Steril 34: 99-105.

Quigley, M.E., Yen, S.S.C. (1980) The role of endogenous opiates on LH secretion during the menstrual cycle. J Clin Endocrinol Metab 51: 179-184.

Ramsey, E.M. (1977) Vascular anatomy. In Wynn, R.M.(ed), Biology of the uterus, Plenum press, New York, pp. 59-76.

Rapoff, M.A., & Barnard, M.U. (1986) Compliance with Paediatric medical regimens. In: Sackett DL, Haynes RB, eds. Compliance with therapeutic regimens. Baltimore, John Hopkins University Press, pp 73-98.

Rapoff, M.A. (1989) Compliance with treatment regimens for paediatrics rheumatic disease. Arthritis Care Res 2:S40-47.

Raskin, A.A. (1961) Comparison of acceptors and resistors of drug treatment as an adjunct to psychotherapy. J Consult Psychol. 25: 366.

Rasmussen, D.D., Liu, J.H., Yen, S.S.C. (1983) Endogenous opioid regulation of the GnRH release from the human medio-basal hypothamus in vitro. J Clin Endocrinol Metab 57: 881-886.

Ravindranath, N., Little-Ihrig, L., Fairchild-Benyo, D., Zeleznik, A.J. (1992) Role of LH in the expression of Cholesterole side-chain cleavage Cytochrome P450 and 3β-hydroxysteroid dehydrogenase, Δ^{5-4} isomerase messenger ribonucleic acids in the primate corpus luteum. Endocrinology. 131: 2065-2070.

Raymond, E.G., Lovely, L.P., Chen-Mok, M., Seppala, M., Kurman, R., Lessey, B.A. (2000) Effect of the Yuzpe regimen of emergency contraception on markers of endometrial receptivity. Hum Reprod 15: 2351-2355.

Revelli, A., Massobrio, M., Tesarik, J. (1998) Nongenomic actions of steroid hormones in reproductive tissues. Endocr Rev 19: 3-17.

Rimmer, C., Horga, M., Cerar, V., Alder, E.M., Baird, D.T., Glasier, A. (1992) Do women want a once-a-month pill? Hum Reprod 7: 608-611.

Robker, R.L., Russell, D.L., Yoshioka, S., Sharma, S.C., Lydon, J.P., O'Malley, B.W., Espey, L.L., Richards, J.S. (2000) Ovulation: a multi-gene, multi-step process. Steroids 65: 559-570.

Rosenburg, M.J., Waugh, M.S., Meehan, T.E. (1995a) Use and mis-use of oral contraceptives; risk indicators for poor pill taking and discontinuation. Contraception 51: 283-288.

Rosenberg, M., Waugh, M.S., Long, S. (1995b) Unintended pregnancies and use, mis-use and discontinuation of oral contraceptive. J Reprod Med 40: 355-360.

Rousseau, G.G., Baxter, J.D., Higgins, S.J., Tomkins, G.M. (1973) Steroid induced nuclear binding of glucocorticoid receptors intact hepatoma cells. J Mol Biol, 79: 539-544.

Rovelli, M., Palmeri, E., Bartus, S., Hull, D., Schweizer, R. (1989) Non-compliance in organ transplant recipients. Transplant Proc 21: 833-834.

Rowen, B.G., O'Malley, B.W. (2000) Progesterone receptor co-activators. Steroids 65: 545-549.

Rowlands, S., Kubba, A.A., Guillebaud, J., Bounds, W. (1986) A possible mechanism of action of danazol and an ethinylestradiol/norgestrel combination used as postcoital contraceptive agents. Contraception 33:539-545.

Royston, J.P. (1982) Basal body temperature, ovulation and the risk of conception, with special reference to the lifetimes of sperm and egg. Biometrics 38: 397-406.

Rudd, P. (1979) In search of the gold standard for compliance measurement (editorial). Arch Intern Med 139: 627-8.

Rudd, P., Ahmed, S., Zachary, V., Barton, C., Bonduelle, D. (1991) Anti-hypertensive drug trials: Contributions from medication Monitors. In: Patient compliance in Medical practice and clinical trials. Cramer, J.A., Spilker, B., (eds.). New York: Raven Press, pp 283-299.

Saatcioglu, F., Claret, K., Karin, M. (1994) Negative transcriptional regulation by nuclear receptors. Semin Cancer Biol 5: 347-359.

Sackett, D.L. (1976) Introduction. In: Sackett, D.L., Haynes, R.B. (eds.) Compliance with therapeutic regimens. Baltimore, John Hopkins University Press, USA. pp 1-6.

Sackett, D.L., Snow, J.C. (1979) The magnitude of compliance and non-compliance. In: Haynes, R.B., Taylor, W.D., Sackett, D.L., (eds.) Compliance in health care. Baltimore, John Hopkins University Press, USA. pp 11-22.

Sadler, S.E., Maller, J.L (1985) Inhibition of Xenopus Laevis adenylate cyclase by progesterone: a novel mechanism of action. Adv Cyclic Nucleotide Protein Phosphorylation Res 19: 179-194.

Sakar, N.N. (2002) Mifepristone: Bio-availability, Pharmacokinetics, and use-effectiveness. Eur J O&G Reprod Biol 101: 113-120.

Salamonsen, L.A., Kovacs, G.T., Findlay, J.K. (1999) Current concepts of the mechanisms of menstruation. Baillieres Clin Obstet Gynaecol 13: 161-179.

Sartorius, C.A., Melville, M.Y., Hovland, A.R., Tung, L,, Takimoto, G.S., Horwitz, K.B. (1994) A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. Mol Endocrinol 8:1347-1360

Schaison, G., George, M., Lestrat, N., Reinburg, A., Baulieu, E.E. (1985) Effects of the antiprogesterone steroid RU 486 during the mid-luteal phase in normal women. J Clin Endocrinol Metab 61: 484-9.

Schatz, F., Markiewicz, L., Gurpide, E. (1986) Hormonal effects of PGF 2α output by cultures of epithelial and stromal cells in human endometrium. 24: 279-301.

Schenken, R.S., Werlin, L.B., Williams, R.F., Prihoda, T.J., Hodgen, G.D. (1986) Histological and hormonal documentation of the luteinized unruptured follicle syndrom. Am J Obstet Gynecol 154: 839-847.

Schiphorst, L.E.M., Collins, W.P., Royston, J.P. (1985) An estrageon test to determine the times of potential fertility in women. Fertil Steril. 44: 328-334.

Schmidt-Matthiesen, H. (1963) Vaskularisierung. In Schmidt-Matthiesen, H. (ed.), Das Normale Menschliche Endometrium. Thieme, Stuttgard, pp.225-244.

Schreiber, J., Nakamura, K., Erickson, G. (1980) Progestins inhibit FSH-stimulated steroidogenesis in cultured rat granulosa cells. Mol Cell Endocrinol 19: 165-173.

Schwartz, D., McDonald, D.P.M., Heuchel, V. (1980) Fecundebility, coital frequency, and the viability of the ova. Popul Stud 34: 397-400.

Seibel, M.M., Smith, D.M., Levesque, L., Borten, M., Taymor, M.L. (1982) The temporal relationship between the luteinizing hormone surge and human oocyte maturation. Am J Obstet Gynecol 142:568-572.

Seif, M.W., Aplin, J.D., Foden, L.J., Tindall, V.R. (1989) A novel approach for monitoring the endometrial cycle and detecting ovulation. Am J Obstet Gynecol 160: 357-362.

Seppala, M., Angervo, M., Koistinen, R., Riittinen, L., Julkunen, M. (1991) Human endometrial protein secretion relative to implantation. Baillieres Clin Obstet Gynaecol 5: 61-72.

Simmons, M.S., Nides, M.A., Rand, C.S., Wise, R.A., Tashkin, D.P. (2000) Unpredictability in deception in compliance with physician prescribed bronchodialator inhaler use in a clinical trial. Chest 118: 290-295.

Sing, E.J., Baccarini, I.M., Zuspan, F.P. (1975) Levels of prostaglandins F2α and E2 in human endometrium during the menstrual cycle. Am J Obstet Gynecol 121: 1003-1006.

Sitruk-Ware, R., Davey, A., Sakiz, E. (1998) Fetal malformation and failed medical termination of pregnancy. Lancet 352:323.

Sheppard, B.I., & Bonner, J. (1980) The development of the vessels of the endometrium during the menstrual cycle. In Diczfalusy, E., Fraser, I.S., and Webb, F.T.G. (eds), Endometrial bleeding and steroidal contraception. pitman press, Bath, UK, pp. 65-77.

Sherman, M.R., Corval, P.L., O'Mallay, B.W. (1970) Progesterone binding components of chick oviduct I. Preliminary characterization of cytoplasmic components. J Biol Chem 245: 6085-6096.

Shortle, B., Dyrenfurth, I., Ferin, M. (1985) Effects of an antiprogesterone agent, RU 486, on the menstrual cycle of the rhesus monkey. J Clin Endocrinol Metab 60: 731-735.

Shoupe, D., Lobo, R.A. (1985) The effects of oestrogen and progestin on endogenous opioid activity in oophorectamized women. J Clin Endocrinol Metab 60: 178-181.

Shoupe, D.,.Mishell, D.R.Jr, Lahteenmaki, P., Heikinheimo, O., Birgerson, L., Madkour, H., Spitz, I.M. (1987) Effects of the antiprogesterone RU 486 in normal women. I single dose administration in the mid luteal phase. Am J Obstet Gynecol 157: 1415-1420.

Shoupe, D.,Mishell, D.R.Jr, Fossum, G., Bopp, B.L., Spitz, I., Lobo, R.A. (1990) Antiprogestin treatment decreases midluteal luteinizing hormone pulse amplitude and primarily exerts a pituitary inhibition. Am J Obstet Gynecol 163: 1982-1985.

Shyamala, G., Schneider, W., Schott, D. (1990) Developmental regulation of murine mammary progesterone receptor gene expression. Endocrinology 126: 2882-2889.

Skafar, D.F. (1991) Differences in the binding mechanism of RU 486 and progesterone to the progesterone receptor. Biochemistry 30:10829-10832.

Slayden, O.D., Brenner, R,M. (1994) RU 486 action after estrogen priming in the endometrium and oviducts of rhesus monkeys (Macaca mulatta). J Clin Endocrinol Metab 78: 440-8

Slayden, O.D., Nayak, N.R., Burton, K.A., Chwalisz, K., Cameron, S.T., Critchley, H.O.D., Baird, D.T., Brenner, R.M. (2001) Progesterone antagonists increase the androgen receptor expression in the rhesus macaque in human endometrium. J Clin Endocrinol Metab. 86: 2668-2679.

Smith, S.K., Abel, M.H., Kelly, R.W., Baird D.T. (1981) Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding. Br J Obstet Gynaeco 88: 434-442.

Smith, S.K., Abel, M.H., Baird, D.T. (1984) Effects of 17 beta-estradiol and progesterone on the levels of prostaglandins F2 apha and E in human endometrium. Prostaglandins 27: 591-597.

Smith, S.K., Kelly, R.W. (1987) The effect of the antiprogestin RU 486 and ZK 98734 on the synthesis and metabolism of prostaglandins F2 alpha and E2 in separated cells from early human deciduas. J Clin Endocrinol Metab 65: 527-534.

Smith, S.K., Kelly, R.W. (1988) The release of PGF2α and PGE2 from separated cells of human endometrium and deciduas. Prostaglandins Leukot. Essent. Fatty Acids 33: 91-96.

Smith, C.L., Nawaz, Z., O'Malley, B.W. (1997) Co-activator and co-repressor regulation of the agonist/antagonist activity of the mixed antioestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11:657-666.

Snijders, M.P., de Goeij, A.F., Debets-Te Baerts, M.J., Rousch, M.J., Koudstaal, J., Bosman, F.T. (1992) Immunohistochemical analysis of oestrogen, and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. J Reprod Fertil. 94: 363-371.

Sopna, J., Matt, K., Schneider, W.H.F. (1975) Study on the action of d-norgestrel as a post-coital agent. Contraception 11: 31-43.

Soules, M.R., Steiner, R.A., Clifton, D.K., Cohen, N.L., Aksel, S., Bremner, W.J. (1984) Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. J Clin Endocrinol Metab 58: 378-382.

Spilker, B. (1991) Methods of assessing and improving patient compliance in clinical trials. In: Cramer, J.A., Spilker, B., eds. Patient compliance in Medical practice and clinical trials. New York: Raven Press, pp37-56.

Spitz, I.M., Bardin C.W. (1993a) Clinical pharmacology of RU 486 – an antiprogestin and antiglucocorticoid. Contraception 48: 403-444.

Spitz, I.M., Croxatto, H.B., Salvatierra, A.M., Heikinheimo, O. (1993b) Response to intermittent RU 486 in women. Fertil & Steril 59: 971-975.

Spitz, I.M., Croxatto, H.B., Robbins, A. (1996) Antiprogestins: mechnism of action and contraceptive potential. Annu Rev Pharmacol Toxicol. 36: 47-81.

Spitz, I.M., Van Look, P.F.A., Bennink, H.J.T.C. (2000) The use of progesterone antagonists and progesterone receptor modulators in contraception. Steroids 65: 817-823.

Stanczyk, F.M. (1994) Metabolism of contraceptive steroids in animals. In: Goldzier J.W., Fotherby, K. (eds) Pharmacology of the contraceptive steroids. New York; Raven Press, pp 53-80.

Steingold, K.A., Matt, D.W., Dua, L., Anderson, T.L., Hodgen, G.D. (1990) Orosomucoid in human pregnancy serum diminishes bioavailability of the progesterone antagonist RU 486 in rats. Am J Obstet Gynecol 162: 523-524.

Sterling, A., Glasier, A.F. (2002) Estimating the efficacy of emergency contraception-how reliable are the data? Contraception 66: 19-22.

Strauss, J.F., Christenson, L.K., Devoto, L., Martinez, F. (2000) Providing progesterone for pregnancy: control of cholesterol flux to the side chain cleavage system. J of Reprod Fertil Supp 55: 3-12.

Stuenkel, C.A., Garzo, V.G., Morris, S., Liu, J.H., Yen, S.S.C. (1990) Effects of antiprogesterone RU 486 in early follicular phase of the menstrual cycle. Fertil & Steril 53: 642-646.

Suzuki, T., Sasano, H., Kimura, N., Tamura, M., Fukaya, T., Yajima, A., Nagura, H. (1994) Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. Hum Reprod 9: 1589-1595.

Swahn, M.-L., Johannisson, E., Daniore, V., de la Torre, B., Bygdeman, M. (1988) The effect of RU 486 administration during the proliferative and secretory phase of the cycle on the bleeding pattern hormonal parameters and endometrium. Hum Reprod 3: 915-921.

Swahn, M.-L., Bygdeman, M., Xing, S., Cekan, S., Masironi, B., Johannisson, E. (1990) The effects of RU 486 administered during the early luteal phase on bleeding pattern hormonal parameters and endometrium. Hum Reprod 5: 402-408.

Swahn, M.-L., Westlund, P., Johannisson, E., Bygdeman, M. (1996a) Effect of post-coital contraceptive methods on the endometrium and the menstrual cycle. Acta Obstet Gynecol Scand 75: 738-744.

Swahn, M.-L., Gemzelle-Danielsson, K., Bygdeman, M. (1996b) Contraception with Antiprogesterone. In: Balliere's Clinical Obstetrics & Gynaecology: Contraception. Glasier, A. (ed.) Balliere Tindall, Oxford, pp. 43 – 55.

Swahn, M.-L., Bygdeman, M., Jun-kang, C., Gemzelle-Danielsson, K., Si, S., Qiu-ying, Y., Pei-juan, Y., Mei-ling, Q., Wei-fang, C. (1999) Once-a-month treatment with a

combination of mifepristone and the prostaglandin analoguemisoprostol. Hum Reprod 14: 485-488.

Swart, P., Swart, A.C., Waterman, M.R., Estabrook, R.W., Mason, J.R. (1993) Progesterone 16α- hydroxylase activity is catalysed by human cytochrome P450 17α-hydroxylase. J Clin Endocrinol Metab 77: 98-102.

Swerdloff, R.S., Odell, W.D. (1969) Serum luteinizing and follicle stimulating hormone levels during sequential and non-sequential contraceptive treatment of eugonadal women. J Clin Endocrinol 29: 157-163.

Tanaka, N., Espey, L.L., Stacy, S., Okamura, H. (1992) Epostane and indomethacin actions on ovarian kallikrein and plasminogen activator activities during ovulation in the gonadotropin-primed immature rat. Biol Reprod 46: 665-670.

Teutsch, G. (1985) Analogues of RU 486 for the mapping of the progestin receptor: synthetic and structural aspects. Baulieu, E.E., Segal, S.J. (eds.) In: The antiprogestin steroid RU 486 and human fertility control. New York; Plenum Press, pp 27-47.

Tesarik, J., Mendoza, C. (1992) Defective function of a non-genomic progesterone receptor as a sole sperm anomaly in infertile patients. Fert Steril 58: 793-797.

Tesarik, J., Carrera,s A., Mendoza, C. (1996) Single cell analysis of tyrosine kinase dependent and independent Ca+2 fluxes in progesterone induced acrosome reactions. Mol Human Reprod 2: 225-232.

Thomas, G.B., Oldham, C.M., Hoskinson, R.M., Scaramuzzi, R.J., Martin, G.B. (1987) Effects of immunization against progesterone on oestrous, cycle length, ovulation rate, luteal regression and LH secretion in the ewe. Aust J Biol Sci 40: 307-313.

Tibbetts, T.A., DeMayo, F., Rich, S., Conneely, O.M., O'Malley, B.W. (1999) Progesterone receptors in the thymus are required for thymic involution during pregnancy and for normal fertility. Proc Natl Acad Sci USA 96:12021-12026.

Too, C.K.L, Bryant-Greenwood, G.D., Greenwood, F.C. (1984) Relaxin increases the release of plasminogen activator, collagenase, and proteoglycanase from rat granulosa cells in vitro. Endocrinology 115: 1043-1050.

Tora, L., Gronemeyer, H., Turcotte, B., Gaub, M.P., Chambon, P. (1988) The N-terminal region of the chicken progesterone receptor specifies target gene activation. Nature: 333: 185-189.

Tremblay, D., Busigny, M., Bonnat, C. (1989) Experimental demonstration in the rat on the role played by human α1-glycoprotein (HAAG) in the nonlinearity of RU 486 pharmacokinetics in women. In: α1-acid Glycoprotein: Genetics, Biochemistry, Physiological function, and pharmacology. Alan R. Liss, New York.

Tremblay, D., Gainer, E., Ulmann, A. (2001) The pharmacokinetics of 750μg levonorgestrel following administration of a one single dose or two doses at 12 or 24 h interval. Contraception 64: 327-331.

Trussell, J., Stewart, F. Guest, F., Hatcher, R.A. (1992) Emergency contraceptive pills: a simple proposal to reduce unintended pregnancies. Fam Plann Perspect 24: 269-273.

Trussell, J., Ellertson, C. (1995) Efficacy of emergency contraception. Fertil Control Rev 4: 8-11.

Trussell, J., Rodriguez, G., Ellertson, C. (1998) New estimates of the effectiveness of the Yuzpe regimen of emergency contraception. Contraception 57: 363-369.

Trussell, J., Raymond, E.G. (1999) Statistical evidence about the mechanism of action of the Yuzpe regimen of emergency contraception. Obstet Gynecol 93: 872-876.

Trussell, J., Rodriguez, G., Ellertson, C. (1999b) Updated estimates of the effectiveness of the Yuzpe regimen of emergency contraception. Contraception 59:147-151.

Truss, M., Beato, M. (1993) Steroid receptors: interaction with deoxyribonucleic acid and transcription factors. Endocr Rev 14: 459-479.

Truss, M., Bartsch, J., Beato, M. (1994) Antiprogesterones prevent progesterone receptor binding to hormone response elements in vivo. Proc. Natl Acad Sci. USA 91: 11333-11337.

Tsai, S.Y., Carlstedt-Duke, J., Weigel, N.L., Dahlman K, Gustafsson JA, Tsai MJ, O'Malley BW. (1988) Molecular interactions of steroid receptor with its enhancer element: Evidence for receptor dimer formation. Cell 57: 1147-1154.

Tseng, L., Gurpide, E. (1975) Induction of human endometrial estradiol dehydrogenase by progestins. Endocrinology 97: 825-833.

Tung, L., Mohamed, M.K., Hoeffler, J.P., Takimoto, G.S., Horwitz, K.B. (1993) Antagonist occupied human progesterone B receptor activates transcription without binding to progesterone response elements and are dominantly inhibited by A receptors. Molecular Endocrinology 7: 1256-1265.

Turner, A.N., Ellertson, C. (2002) How Safe is Emergency Contraception? Drug Saf 25: 695-706.

Uhler, M.L., Leung, A., Chan, S.Y., Wang, C. (1992) Direct effects of progesterone and antiprogesterone on human sperm hyperactivate motility and acrosomal reaction. Fertil Steril 58: 1191-1198.

U.N. (1994) Report of the International Conference on Population and Development (Cairo). New York: UN Publication.

U.N. (1995) Levels, and trends of contraceptive use as assessed in 1994. New York: UN Publications: sales no. E96. XIII.13.

U.N. (1998) World population prospects: The 1998 revision. New York: UN Publication, E.99 XIII.3.

U.N. (1999) World population prospects: The 1999 revision. Volume I: Comprehensive tables. Department for economic and social affairs, population division, United Nations, New York. (Reviewed via Internet).

U.N. (1999a) Levels, and trends of contraceptive use as assessed in 1998. Department for Economic and Social Affairs, population divisions, United Nations, New York. (Reviewed via Internet).

Urquhart, J. (1989) Non-compliance: The ultimate absorption barrier. In: Prescott LF, Nimmo WS, eds. Novel Drug Delivery and its Therapeutic Application. New York, Wiley, pp 127-137.

Ulmann, A., Dubois, C., Philibert, D. (1987) Fertility control with RU 486. Horm Res 28: 274-278.

Van Uem, JF, Hsiu, JG, Chillik, CF. (1989) Contraceptive potentials of RU 486 by ovulation inhibition: I. Pituitary versus ovarian action with blockade of estrogen induced endometrial proliferation. Contraception 35: 433-438.

Vande Wiele, R.L., Bogumil, J., Dyrenfurth, I., Ferin, M., Jewelewicz, R., Warren, M., Rizkallah, T., Mikhail, G. (1970) Mechanisms regulating the menstrual cycle. Recent Progr Hormone Res 26: 63-94

Vargayas, J.M., Marrs, R.P., Kletzky, O.A., Mishell, D.R. (1982) Correlation of ultrasonic measurement of ovarian follicle size and serum oestradiol levels in ovulatory patients following clomiphene citrate for in vitro fertilization. Am J Obstet Gynecol 144: 569-573.

Vegeto, E., Allan, G.F., Schrader, W.T., Tsai, M.J., McDonnell, D.P., O'Malley, B.W. (1992) The mechanism of RU 486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. Cell 69: 703-713.

Vegeto, E., Shabaz, M.M, Wen, D.X., Goldman, M.E., O'Malley, B.W., McDonell, D.P. (1993) Human progesterone receptor A type is a cell and promoter- specific repressor of human progesterone receptor B function. Mol Endocrinol 7: 1244-1255.

Vermesh, M., Kletzky, O.A., Davajan, V., Israel, R. (1987) Monitoring techniques to predict and detect ovulation. Fertil Steril 47: 259-264.

Victor, A., Weiner, E., Johansson, E.D.B. (1976) SHBG: the carrier protein for LNG. J Clin Endocrinol Metab 43: 244-247.

Victor, A., Weiner, E., Johansson, E.D.B. (1977) Relation between SHBG and LNG levels in plasma. Acta Endocrin 86: 430-436.

Vollman, R.F. (1977) The menstrual cycle. Major Probl Obstet Gynecol 7: 1-193.

Walters, M.R., Clark, J.H. (1977) Cytosol progesterone receptor of the rat uterus: assay and receptor characteristics. J Steroid Biochem, 8:1137-1144.

Wang, H., Critchley, H.O.D., Kelly, R.W., Shen, D., Baird, D.T. (1998) Progesterone receptor subtype B is differentially regulated in human endometrial stroma. Mol Hum Reprod 4: 407-412.

Warren, R.J., Fotherby, K.(1974) Radioimmunoassay of norethisterone and norgestrel. J Endocrinol 62: 605-618.

Waterhouse, D.M., Calzone, K.A., Mele, C., Brenner, D. (1993) Adherence to oral tamoxifen: A comparison of patient self-report, pill counts and microelectronic monitoring. J Clin Oncol 11: 1189-1197.

Webb, A.M.C., Russell, J., Elstein, M. (1992) Comparison of Yuzpe regimen, Danazol, and mifepristone (RU 486) in oral post-coital contraception. BMJ 305: 927-931.

Webster, N.J.G., Green, S., Jim, J.-R., Chambon, P. (1988) The hormone binding remains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. Cell 54: 199-207.

Weibe, H., Morris, C. (1984) Effect of an oral contraceptive on adrenal, and ovarian androgenic steroids. Obstet Gynecol 63: 12-14.

Weigel, NL, Beck, CA, Estes, PA. (1992) Ligands induce conformational changes in the carboxy-terminus progesterone receptors which are detected by a site-directed anti-peptide monoclonal antibody. Molecular Endocrinology 6: 1585-1597.

Weigel, N.L. (1993) Overview and background : Mechanism of action of antiprogestins. In: Clinical applications of mifepristone (RU 486) and other anti-progestins. Donaldson, M.S., Dorflinger, L., Brown, S.S., Benet, L.Z., eds. Natl Acad Press, Washington pp.120-138.

Weigel, N.L. Receptor phosphorylation. (1994) In: Tsai, M.J., O'Malley, B.W., eds. Mechanism of steroid hormone regulation of gene transcription. Austin, R.G. Landes Co., pp. 93-110.

Weiner, E., Johansson, E.D.B., Wide, L. (1976) Inhibition of the positive feedback of oestradiol during treatment with subcutaneous implants of d-norgestrel. Contraception 13: 287-298.

Weiner, E., Victor, A., Johansson, E.D.B. (1976) Plasma levels of LNG after oral administration. Contraception 14: 563-570.

Wheble, A.M., Street, P., Wheble, S.M. (1981) Contraception: Failure in practice. Br J Fam Plann 7: 41-44.

W.H.O. (1983) Temporal relationship between indices of the fertile period. Fertil Steril 39: 647-655.

W.H.O. Task Force on methods for the determination of the fertile period (1980): Temporal relationships between ovulation and defined changes in the concentrations of

plasma oestradiol-17β, luteinizing hormone, follicle-stimulating hormone and progesterone. Am J Obstet Gynecol 138:383-387.

W.H.O. laboratory manual for the examination of human semen and sperm. (1992) Chapter 3; Sperm-cervical mucus interaction. Cambridge University Press, 3rd Edition, pp 28-53.

W.H.O. Task Force on postovulatory Methods of Fertility Regulation. (1998) Randomised controlled trial of LNG versus the Yuzpe regimen of combine oral contraceptives for emergency contraception. Lancet 352: 428- 433.

W.H.O. Task Force on Postovulatory Methods of Fertility Regulation. (1999) Comparison of three single doses of mifepristone as emergency contraception: A randomised trial. Lancet 353: 697-702.

Wilcox, A.J., Weinburg, C.R., O'Conner, J.F., (1988) Incidence of early loss of pregnancy. N Engl J Med 319: 189-194.

Wilcox, A.J., Weinburg, C.R., Baird, D.D. (1995) Timing of sexual intercourse in relation to ovulation: Effects on the probability of conception, survival of the pregnancy and the sex of the baby. N Engl J Med 333: 1517-1521.

Wilcox, A.J., Weinburg, C.R., Baird, D.D. (1998) Post ovulatory aging of the human oocyte and embryo failure. Hum Reprod 13: 394- 397.

Wildt, L., Hutchison, J.S., Marshall, G., Phol, C.R., and Knobil, E. (1981) On the site of action of progesterone in the blockade of the estradiol-induced gonadotrophin discharge in the rhesus monkey. Endocrinology 109: 1293-1294.

Wolf, J.P., Hsiu, J.G., Anderson, T.L. (1989) Non-competitive anti-estrogenic effect of RU 486 in blocking the estrogen-stimulated luteinizing hormone surge and the proliferative action of estradiol on endometrium in castrate monkeys. Fertility and Sterility 52: 1055-1060.

Wright, E.C. (1993) Non-compliance- or how many aunts has Matilda? Lancet 342: 909-913.

Yen, S.S.C. (1991) The human menstrual cycle: neuroendocrine regulation. In: Reproductive endocrinology, pathophysiology and clinical management. Yen, S.S.C., Jaffe, R.B. (eds.) W.B. Sanders Co., Harcourt Brace Jovanovich Inc. Philadelphia, London, pp. 1-13.

Yki-Jarvinen, H., Wahlstrom, T., Seppala, M. (1985) Human endometrium contains relaxin that is progesterone dependent. Acta Obstet Gynecol Scand 64: 663-665.

Yong, E.L., Glasier, A., Ledger, W., Caird, L., Beattie, G., Thong, J., Baird, D.T. (1992) Effect of cyclofenil on hormonal dynamics, follicular development and cervical mucus in normal and oligomenorrhoeic women. Hum Reprod 7: 39-43.

Yoshinaga, K. (1978) Cyclic hormone secretion by mammalian ovary. In: Jones, R.E. (ed), The vertibrate Ovary. New York, Plenum Press, pp 691-729.

Yuzpe, A.A., Lancee, W.J. (1977) Ethinyl estradiol and dl-norgestrel as postcoital contraceptive. Fertil Steril 28: 932-936.

Zander, J. (1954) Progesterone in human blood and tissues. Nature 174; 406-407.

Zeimet, A.G., Muller-Holzner, E., Martha, C., Daxenbichler, G. (1994) Immunohistochemical vs. biochemical receptor determination in the normal and tumorous tissues of the female reproductive tract and the breast. J Steroid Biochem Mol Biol. 49: 365-372.

Zuliani, G., Colombo, U.F., Molla, R. (1990) Hormonal post-coital contraception with ethinylestradiol-norgestrel combination and two Danazol regimens. Eur J Obstet Gynaecol Reprod Biol 37: 253-260.

Appendix

Published Papers

The following papers have been published based on the text of this thesis;

Original Articles.

1. **Hapangama, D.K.,** Glasier, A.F., Baird, D.T. (2001) The effects of peri-ovulatory administration of Levonorgestrel on the menstrual cycle. Contraception 63:123-129.

2. **Hapangama, D.K.,** Brown, A., Glasier, A.F., Baird, D.T. (2001) Feasibility of administering mifepristone as a once a month pill.
   Hum Reprod 16(6): 1145-1150.

3. **Hapangama, D.K.,** Glasier, A.F., Baird, D.T. (2001) Non-compliance in a group of women using a novel method of contraception.
   Fertil & Steril 76(6): 1196-1201.

4. **Hapangama, D.K.,** Critchley, H.O.D., Henderson, T., Baird, D.T. (2002) Mifepristone Induced Vaginal Bleeding Is Associated With Increased Immunostaining For Cyclooxygenase 2 and Decrease In Prostaglandin Dehydrogenase In Luteal Phase Endometrium.
   J Clin Endocrinol & Metab. 87(11): 5229-5234.

ELSEVIER

Contraception

Contraception 63 (2001) 123–129
Original research article

The effects of peri-ovulatory administration of levonorgestrel on the menstrual cycle☆

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Abstract

Levonorgestrel (LNG) 0.75 mg administered 12 h apart within 72 h of unprotected coitus, is an established method of emergency contraception (EC). The mechanism of action of LNG used in this manner is unknown. We administered LNG 0.75 mg twice immediately before ovulation, to test the hypothesis that LNG acts as an emergency contraceptive by abolishing the pre-ovulatory lutenizing hormone (LH) surge and thereby delaying ovulation. Twelve women took LNG on or before the day of the first significant rise in urinary LH in 12 cycles. In four women, the LH peak and the onset of next menses were significantly delayed (delay of 16.8 days (SD ± 8.7) from the day of mean LH peak in placebo cycles). One woman did not ovulate at all, despite a normal LH peak and cycle length. In the remaining eight women, LNG did not affect ovulation or the cycle length, but the length of the luteal phase and the total luteal phase LH concentrations were significantly reduced. We suggest that LNG acts as an emergency contraceptive by other mechanisms as well as delaying the LH surge and interfering with ovulation.

Keywords: Emergency contraception; Levonorgestrel; Mechanism of action

1. Introduction

Levonorgestrel (LNG) 0.75 mg administered twice with the two doses 12 h apart has been shown to be an effective method of emergency contraception (EC) when used within 72 h of unprotected intercourse [1,2]. Although the regimen is now licensed in the UK, USA, and throughout much of Europe and is widely regarded as the emergency contraceptive method of choice [3], the mechanism of action remains unknown. The mechanism of action of the Yuzpe regimen of emergency contraception (ethinyl estradiol 100 mcg and 0.5 mg LNG, two doses 12 h apart [4–6], is also incompletely understood but there is good evidence that it delays or inhibits ovulation in at least some cycles [5]. In the WHO study, the efficacy of both LNG and the Yuzpe regimen decreased with time after intercourse [7] and both regimens had a similar effect on the timing of the subsequent menses, suggesting that the mechanism of action of the two regimens may be similar. It has also been shown that large amounts of synthetic progestogens abolish the mid-cycle lutenizing hormone (LH) surge leading to anovulation and delaying the onset of the subsequent menses [8–11].

To test the hypothesis that it acts as a post-coital agent by abolishing the pre-ovulatory LH surge and by delaying ovulation, we administered LNG 0.75 mg twice to 12 healthy female volunteers in the fertile period (immediately before ovulation) of the menstrual cycle and investigated the effects on the timing of ovulation and of the next menses, bleeding patterns; ovarian activity, and LH concentrations.

2. Materials and methods

This was a prospective, randomized, double-blind, cross-over study undertaken in one center. Twelve healthy women (mean age 33.3; range 26–41 years) with regular cycles (mean 27.6 days; range 25–30 days) and mean BMI 25.7 (range 20–34) were recruited. They were all using a reliable non-hormonal method of contraception or were abstinent

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during the study. All subjects gave written informed consent for participation in the study, which was approved by the Lothian Research Ethics Committee.

A method was sought to provide a convenient means of identifying the fertile period prior to ovulation, and thereby to time the administration of LNG. Unipath (Bedford, UK) had developed a technology that can be used in the home to monitor changes in urinary hormones [12]. This monitoring system comprises disposable test sticks and a hand-held monitor, which together are used to detect changes in the levels of oestrone-3-glucuronide (E3G), a urinary metabolite of oestradiol, and LH to indicate the potentially fertile days leading up to ovulation.

The monitor optically measures the intensity of the lines that form on the test sticks after sampling, and the system will delineate three levels of fertility (Low, High, and Peak Fertility) according to the optical signal changes detected. Low Fertility will be displayed from day 1 of the cycle until the hormone levels rise above the baseline levels. A change from Low to High Fertility is triggered by detection of elevated E3G levels, to concentrations typically between 20 and 30 ng/mL. The change from High to Peak Fertility is triggered by the detection of an LH surge, typically with a concentration higher than 30 IU/L. Peak fertility is displayed on the day of the LH surge and on the following day.

Each woman was studied during four cycles and was issued a monitor at the beginning of the study. Subjects were asked to use the monitor according to instructions, and familiarized themselves with the monitors by using it during a pre-study cycle to identify the days of high fertility and the day of the LH surge. They also recorded days of vaginal bleeding.

Data from the pre-study cycle was used to predict the timing of the LH surge and of the fertile phase during the study cycles and thereby to predict when treatment should be administered. The three study cycles followed immediately after the pre-study cycle. Six subjects were randomly assigned to treatment arm A and received LNG in the first study cycle and placebo in the third study cycle. The remaining six subjects were randomized to treatment arm B and received placebo in the first study cycle and LNG in the third study cycle. The second study cycle was a washout phase for all women during which time they also received placebo tablets.

The randomization list was produced using SPSS Rv.Bernouilli function such that each study number was randomly assigned to either treatment arm A or treatment arm B with the same probability, i.e. 0.5.

LNG and placebo were visually identical and were prepacked. Each subject collected a sample of early morning urine daily from the first day of the first study cycle until and including the first day of the menstrual bleed signaling the end of the last study cycle. Samples were frozen and later assayed in batches (with all samples from one subject assayed in a single batch) for measurement of urinary LH, E3G, and pregnanediol-3-glucuronide (P3G).

Quantitative assessment of urinary LH was performed using an LH MAIAclone kit (BIOSTAT-DIAGNOSTICS, Stockport, Cheshire, UK). This method incorporates two high-affinity monoclonal antibodies into an immunoradiometric assay system and offers a working range of 1.5–200 mIU/mL. Urinary P3G was measured using a direct enzyme immunoassay (working range 0.25–32 mmol/L), while direct immunoassay was used to measure E3G levels (working range 8.36–2140 nmol/L. Intra-assay coefficients of variation were 6% for E3G, 10% for P3Gl, and 3% for LH [13]. Geometric means of daily replicates were divided by the respective daily creatinine concentration to correct for variations in the dilution of the urine specimen.

During study cycles 1 and 2, women were asked to take the study medication on the first day of High Fertility as identified by the monitor. However, by the third study cycle, the variation in the number of high fertile days (range 0–8 days) meant that the monitor could not be used to administer medication on LH-2 in every cycle. Therefore, we had to adopt a different method of calculating the anticipated day of the LH peak for each cycle based on the monitor information from the previous cycles (including the pre-study cycle). Hence, in the third study cycle, the medication was taken 2 days prior to the anticipated day of the LH peak. In all cycles the first tablet was taken at 1100 h and the second at 2300 h. A sample of venous blood was collected 5–7 days after treatment, stored and later assayed for progesterone using Coat-A-Cont solid-phase radioimmunoassay. The subjects kept a daily record of all vaginal bleeding experienced during the four cycles, the fertility status information displayed each day on the monitor LCD and the days on which the study medications were taken.

2.1. Statistical analysis

We calculated that a total of six subjects in each of the two treatment arms would give more than 90% power to detect a delay of menses of >5 days in 95% of cycles.

Preliminary analysis was performed to determine whether parametric tests were appropriate for analysis of the data. Outlying data points were investigated while the treatment was still blinded. The period effect and interaction between treatment and period effect were tested (two-sample *t* test) before progressing to testing of a treatment effect and was non-significant. Comparisons between LNG versus placebo cycles was tested by paired *t* test.

For the purpose of the study the following definitions based on the quantitative data were created.

A significant delay in the onset of next menses: Delay of 5 or more days from the expected onset of menses (based on the mean cycle length for the 2 placebo cycles).

The LH peak was defined as a significant rise in urinary LH concentration, with a minimum of 50% rise above the average baseline level for 4 preceding days and which remained elevated for a minimum of 3 days.

The first day of the LH peak was defined as the day of the

Table 1
Timing of LH peak, predicted day of the LH peak and timing of LNG in treatment cycles for all subjects

Subjects	Day of the LH peak (LH > 50%)				Predicted day of the LH peak in the treatment cycle	Timing of LNG in relation to the day of LH peak in the treatment cycle	Timing of LNG in relation to the predicted day of the LH peak
	Pre-study cycle	Placebo	Placebo (washout)	Treatment			
S101	12	13	9	10	11	−1	−2
S102	M	20	19	21	19.5	−1	+0.5
S103	12	27[c]	9	11	9	−1	+1
S104	M	11	12	12	11.5	0	+0.5
S107	17	14	14	13	14	−1	−2
S111	12	11	15	12	13	−1	−4
S112	11	11	12	13	11.5	−5	−3.5
S105[a]	14	13	15	14	14	−2	−2
S106[b]	14	14	12	23	13	−11	−2
S108[b]	17	18	18	25	14	−13	−2
S109[b]	14	10	10	38	10	−31	−3
S110[b]	18	18	££	40	18	−25	−3

[a]The woman with normal LH peak but no significant rise in pregnanediol in the luteal phase = anovulatory cycle.
[b]Women who had a delay of the LH peak by >5 days.
[c]Excluded, unusually delayed ovulation.
££ = excluded, no daily urine available.
M = LH peak not detected by the monitor.

first significant rise (>50% above the baseline) seen at the beginning of the LH peak.

Retrospectively predicted first day of the LH peak for the treatment cycles was the calculated mean of the first day of LH peak in the two placebo cycles.

A significant delay in the first day of the LH peak: Delay of 5 or more days from the expected first day of the LH peak in the treatment cycle (based on the mean first day of the LH peak during the 2 placebo cycles).

Luteal phase: time from the day after the first day of the urinary LH peak (LH+1) until, and including, the day before the first day of the next menses.

Follicular phase: time from the first day of the menses until the day of the first significant rise in urinary LH (LH+0) inclusive.

3. Results

A total of 48 menstrual cycles were studied—12 pre-study cycles and 36 study cycles. Data from daily urine samples were available for 34 out of the 36 study cycles. In one woman (S103) the first study cycle, which was a placebo cycle, was prolonged (41 days) as a consequence of a delay in ovulation. Her usual cycle length was 25 days, this cycle was excluded from the analysis. In a second woman (S110) there were no daily urine samples available from the washout cycle as she was abroad on holiday. Therefore, daily urine samples were only available for this subject from two study cycles (the treatment cycle and one placebo cycle).

3.1. Timing of administration of LNG

Six women took LNG in the first study cycle and six took it during the third study cycle. During the first study cycle, 10 women took the tablet (either placebo or LNG) on the first day of High Fertility as indicated by the monitor. The remaining 2 women took the tablet on the first day of the urinary LH peak because the monitor failed to identify any high fertile days prior to the LH surge.

The variation in the number of High Fertile days (0–8 days) declared by the monitor meant that the system could not be used to predict LH-2 in every cycle. Therefore, for cycle 3 we calculated the anticipated first day of the urinary LH peak from the information gathered from the pre-study cycle and study cycles 1 and 2 for each woman and instructed subjects to take the tablet 2 days before the anticipated day of the LH peak.

After completion of the study, we retrospectively calculated the predicted first day of the urinary LH peak for every treatment cycle based on the mean first day of the LH peak in the two placebo cycles. When we applied this retrospectively predicted definition to all 12 treatment cycles, the day of taking LNG ranged from 4 days before until 1 day after the first day of the anticipated LH peak. However, in reality, LNG was never taken after the first significant rise in urinary LH concentrations in any treatment cycle. The timing of the LH peak in each of the four cycles, the predicted day of the LH peak day and the timing of LNG and placebo treatment in relation to the start of the actual LH peak are shown in Table 1.

Table 2
Mean length of placebo and treatment cycles

Mean cycle length	Treatment cycles	Placebo cycles
All cycles, n = 12[a]	32.17 (SD ± 3.36)	26.33 (SD ± .42)
N = 4, Delay of >5 days[b]	42.75 (SD ± 8.42)	27.13 (SD ± 1.84)
N = 8, remaining cycles[c]	24.88 (SD ± 2.1)	26.13 (SD ± 1.69)

[a] p = 0.14.
[b] p = 0.04.
[c] p = 0.12.

3.2. Cycle length

Treatment with LNG in the pre-ovulatory period significantly prolonged by 5 days or more the mean cycle length in four women (33% of the sample, Table 2). All 4 women reported vaginal spotting 2 to 3 days after taking LNG and they all had a second episode of vaginal bleeding between 9 and 16 days after the delayed LH peak. In the remaining eight women, there was no significant difference in cycle length between treatment and placebo cycles. One of this group of women, however, reported light vaginal bleeding starting a week after taking LNG, the bleeding continued until she started what she regarded as a normal menstrual period which followed a fall in urinary pregnanediol levels. Her hormone profile during the treatment cycle followed a normal pattern.

3.3. The first day of the LH peak

In the four women with long cycles, LNG appeared to abort the LH peak and a subsequent LH peak occurred 7 to 16 days later, followed by a normal rise in urinary pregnanediol. The urinary hormone profile of one woman (S110) is illustrated in Fig. 1. In the remaining eight women, LNG did not affect the timing of the LH peak when taken immediately before ovulation. Fig. 2b illustrates the hormone profile in one of these women (S101).

3.4. Length of the luteal phase

In all 12 volunteers the luteal phase was significantly shortened following treatment with LNG as compared with the placebo cycles (mean length 11.5 days [SD ± 1.8] vs. 12.9 days [SD ± 2.5] p = 0.005, Table 3).

3.5. The effect on total LH during the luteal phase

Daily urinary LH concentrations were summated from the first day of the LH peak (LH+0) up to the day before the first day of the next menses to give a value for total LH concentrations. The 8 women in whom pre-ovulatory LNG did not affect the cycle length, showed a significant (p = 0.01) decrease in total LH in the treatment cycles (18.7 U/mmoL,

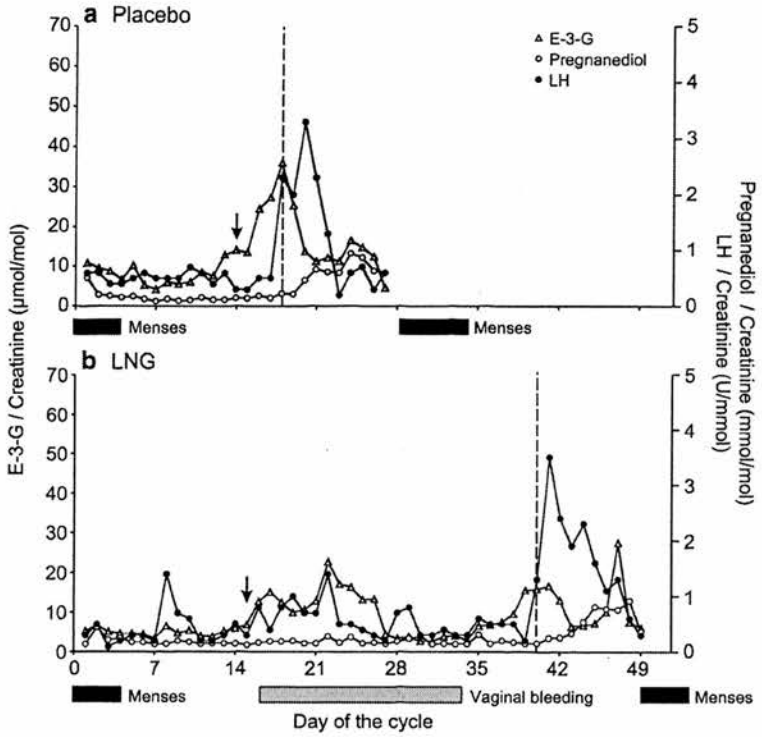

Fig. 1. Daily levels of LH (●) oestrone-3-glucuronide (E3G) (△), and pregnanediol (○), in urine relative to the cycle day. (a) Placebo cycle of a woman (S110) (b) Treatment cycle of the same woman (S110) showing significantly prolonged cycle following pre-ovulatory LNG. ↓, Day of taking LNG or placebo tablet; – – – –, day of the LH surge

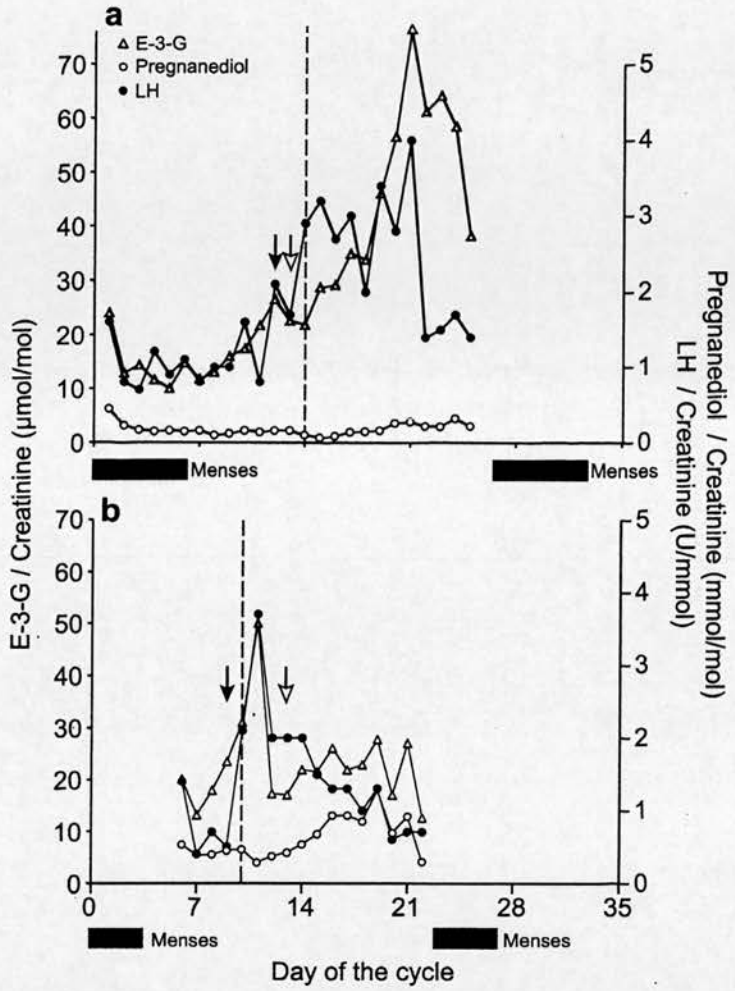

Fig. 2. Daily levels of LH (●), oestrone-3-glucuronide (E3G) (△), and pregnanediol (○), in urine during the treatment cycles. (a) Of the woman (S105) with no significant rise in pregnanediol following LNG. (b) Of a women (S101) with apparently normal cycle length. ↓, Day of taking LNG; ⇓, day of the LH surge in the placebo cycle; – – –, day of the LH surge.

SD ± 8.9) as compared with the placebo cycles (27.1 U/mmoL, SD ± 13.6). In contrast, among the 4 women with significantly longer cycles after taking LNG, there was no difference in the total LH secretion in the luteal phase (mean total LH = 18.8 mIU/mL, SD ± 8.3; versus mean total LH for placebo cycles of 17.8 U/mmoL, SD ± 2.8; p = 0.81).

3.6. Effect on pregnanediol in the luteal phase

The sum of daily pregnanediol concentrations in the luteal phase (from LH+1 onwards up to the day before the first day of next menses) was compared during the treatment and placebo cycles was employed to indirectly assess the function of the corpus luteum.

In one woman (S105) after taking LNG, there was no significant rise in urinary pregnanediol levels (>0.5 mmol/mol creatinine as expected in the mid-luteal phase) despite an apparently normal LH peak. The mid-luteal serum progesterone level during this treatment cycle was consistent with an anovulation (<5 nmol/L) (Fig. 2a). In the remaining 11 subjects, the total values of pregnanediol did not show a significant difference between placebo or treatment cycles.

4. Discussion

Evaluation of daily hormone concentrations confirmed that all 12 women in our study took LNG before the LH peak, and presumably ovulation. Seven women had apparently normal ovulatory cycles after taking LNG. Five of them took LNG on the day before the LH surge and one on the day of the surge. It is possible that the timing of LNG in these women was "too late" to influence an event already well underway. However, in the four women in whom the LH peak and ovulation was delayed, the LNG was taken within 3 days of the predicted LH peak. One woman did not ovulate at all despite having an LH surge 2 days after taking LNG.

Table 3
Length of the luteal phase (n = 12)

Patient	Treatment cycle	Mean for placebo cycles
101	12	13.5
102	8	8.5
103	14	14
104	13	15.5
107	13	14
111	12	13.5
112	10	14
105	11[b]	13.5
106[a]	12	12
108[a]	10	11.5
109[a]	13	16.5
110[a]	9	9

[a]Women who had a delay of the LH peak by >5 days
[b]The time from the LH surge to the next menses in the woman with an anovulatory cycle, but a normal LH peak after LNG (Fig 2a)

It is apparent that different women respond differently to the administration of LNG. The effects observed may be related to administering LNG at a specific stage in follicular development. Even though the timing of the LNG in relationship to the onset of the LH surge did not appear to be different between women in whom ovulation was affected and those in whom it was not, it would be naïve to accept that our predicted day of the LH peak based on information gathered in two cycles was always accurate. A more detailed study employing daily serum levels of gonadotrophins and steroid hormones and ultrasound scans to correlate follicular size and maturity to the timing of the administration of LNG might provide an explanation.

The 7 women, who apparently ovulated normally, had a reduced total luteal LH and a shortened luteal phase. Basal levels of LH are essential for the normal secretory function of the corpus luteum [14]. In the mid-luteal phase, LH inhibition by the administration of GnRH antagonists consistently results in luteolysis in women as well as in non-human primates [15–17]. There are no direct ways of measuring whether the function of the corpus luteum is compatible with the establishment of pregnancy. Although there was no significant difference in the urinary pregnanediol levels after LNG, it is possible that the shortened luteal phase observed was a consequence of reduced total LH and may have a contragestive effect.

If LNG acts as an emergency contraceptive only by interfering with ovulation, the expected efficacy should fall below 42% (5 of 12 women). Ho and colleagues [1] reported that LNG reduced approximately 60% of the expected number of pregnancies (estimates were based on the table of probabilities of pregnancy at different cycle days by Dixon et al. [18]). LNG fared better in the WHO study [2] with overall 85% reduction of expected number of pregnancies (the analysis of the prevented fraction was based on the modified Wilcox estimates of conception probabilities [19]).

Both studies reported effectiveness against estimates based on historical data. The fertile period was determined on the assumption that ovulation occurred 14 days before the next expected menses. The validity of using these estimates directly relies on the accuracy of reported menstrual cycle data. Women do not regularly keep records of their menses, and by and large the sexual intercourse responsible for requesting emergency contraception is unpremeditated. Reporting errors are common and the estimates can be inaccurate. In addition, other factors such as biologic variability of the day of the ovulation and the length of the luteal phase, factors affecting the probability of pregnancy unrelated to the timing of intercourse, and heterogeneity among couples in fecundability can distort the estimated number of pregnancies. In a study comparing the efficacy of the Yuzpe regimen of EC with a single dose of mifepristone [20], there were frequent discrepancies among subjects between the stage of the cycle as estimated from the date of the LMP and that suggested by circulating concentrations of progesterone. There has never been a placebo-controlled trial of EC. Thus, it is possible that the genuine effectiveness of LNG as an emergency contraceptive is less than 42%.

One woman (S103) in our study showed a delayed LH peak (on day 27) during a placebo cycle and subsequently the length of that cycle was prolonged to 41 days (her usual cycle length was 25 days). In contrast to the four women who experienced similar prolongation of the cycles after taking LNG, this woman did not report any intermenstrual vaginal bleeding. Although we excluded this cycle from our analysis, similar spontaneously occurring prolonged cycles (with delayed ovulation), can influence the results of studies into emergency contraception.

The discrepancy noted in the estimated effectiveness of LNG and the prevalence of ovulation delay or inhibition in our study may be due to mechanisms of action other than interference with ovulation. Our study was not designed to investigate the other possible mechanisms by which LNG works. However, one woman in our study reported slight vaginal bleeding after taking LNG with an apparently normal LH peak, cycle length and hormone profile. This may suggest an additional effect of LNG on the endometrium [21–24]. Nevertheless, the question remains as to whether similar alterations occur in the endometrium after taking the emergency contraceptive regimen of LNG, and whether these changes are sufficient to prevent implantation and account for the observed contraceptive efficacy of LNG. The effect of progestogens on cervical mucus and on the cervix is well documented and this is thought to be the main mechanism by which the progestogen-only pill exerts its antifertility action [25–27]. However, even if LNG has an effect on cervical mucus, which interferes with sperm penetration, that action is unlikely to prevent pregnancy when taken some 12–72 h after coitus.

The reason for using the monitor to time the administration of LNG or placebo was to avoid having to subject the volunteers to regular blood samples and ultrasound scans.

However, due to the variability in the number of high

fertile days declared prior to the LH surge, greater reliance had to be placed on calendar calculations to predict the LH surge.

In conclusion, we suggest that LNG taken immediately before ovulation acts as an emergency contraceptive by delaying or preventing ovulation. Other plausible actions of LNG including the retardation of the endometrium, interfering with sperm motility and altering cervical mucus may be important, and need to be explored further.

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References

[1] Ho PC, Kwan MSW. A prospective randomized comparison of levonorgestrel with the Yuzpe regimen in post-coital contraception. Hum Reprod 1993;8:389–92.

[2] WHO Task Force on Postovulatory Methods of Fertility Regulation. Randomised controlled trial of LNG versus the Yuzpe regimen of combined oral contraceptives for emergency contraception. Lancet 1998;352:428–33.

[3] Guillebaud J. Time for emergency contraception with levonorgestrel alone. Lancet 1998;352:416

[4] Yuzpe AA, Lancee WJ. Ethinyl estradiol and dl-norgestrel as post-coital contraceptive. Fertil Steril 1977;28:932.

[5] Swahn M-L, Westlund P, Johannisson E, Bygdeman M. Effect of post-coital contraceptive methods on the endometrium and the menstrual cycle. Acta Obstet Gynecol Scand 1996;75:738–44.

[6] Raymond EG, Lovey LP, Chen-Mok M, Seppala M, Kuran R, Lessey BA. Effect of the Yuzpe regimen of emergency contraception on markers of endometrial receptivity. Hum Reprod 2000;15:2351–5.

[7] Piaggio G, Von Hertzen H, Grimes DA, Van Look PFA. Timing of emergency contraception with levonorgestrel or the Yuzpe regimen. Lancet 1999;353:721.

[8] Kesseru E, Garmendia F, Westphal N, Parada J. The hormonal and peripheral effects of D-norgestrel in postcoital contraception. Contraception 1974;10:411–24.

[9] Landgren BM, Dada O, Aedo AR, Johannisson E, Diczfalusy E. Pituitary, ovarian, and endometrial effects of 300 μg LNG administered on cycle days 7 to 10. Contraception 1990;41:569–81.

[10] Craft I, Foss GL, Warren RJ, Fotherby K. Effect of norgestrel administered intermittently on pituitary ovarian function. Contraception 1975;12:589–98.

[11] Sopna J, Matt K, Schneider WHF. Study on the action of D-norgestrel as a post-coital agent. Contraception 1974;11:31–43.

[12] Bonnar J, Flynn A, Freundl G, Kirkman R, Royston P, Snowden R. Personal hormone monitoring for contraception. Br J Fam Plan 1999; 24:128–34.

[13] Yong EL, Glasier A, Ledger W, et al. Effect of cyclofenil on hormonal dynamics, follicular development and cervical mucus in normal and oligomenorrhoeic women. Hum Reprod 1992;7:39–43.

[14] Vande Wiele RL, Bogmil J, Dyrenfurth I, et al. Mechanisms regulating the menstrual cycle in women. Recent Progr Hormone Res 1970;26:63–94.

[15] Hall JE, Bhatta N, Adams JM, Rivier JE, Vale WW, Crowley WF. Variable tolerance of the developing follicle and corpus luteum to GnRH-releasing hormone antagonist induced gonadotropin withdrawal in the human. J Clin Endo Metabol 1991;72:993–1000.

[16] Hutchison JS, Zeleznik AJ. The rhesus monkey corpus luteum is dependent on pituitary gonadotropin secretion throughout the luteal phase of the menstrual cycle. Endocrinology 1984;115:1780–6.

[17] Ravindranath N, Little-Ihrig L, Fairchild Benyo D, Zeleznik AJ. Role of LH in the expression of cholesterol side-chain cleavage, cytochrome P450 and 3(-hydroxysteroid dehydrogenase, (5-4 isomerase messenger ribonucleic acids in the primate corpus luteum. Endocrinology 1992;131:2065–70.

[18] Dixon GW, Schlesselman JJ, Ory HW, Blye RP. Ethinyl estadiol and conjugated estrogens as postcoital contraceptives. JAMA 1980;244: 1336–9.

[19] Trussell J, Rodriguez G, Ellertson C. New estimates of the effectiveness of the Yuzpe regimen of emergency contraception. Contraception 1998;57:363–9.

[20] Glasier AF, Thong KJ, Dewar M, Mackie M, Baird DT. Mifepristone (RU 486) compared with high-dose estrogen, and progestogen for emergency postcoital contraception. N Engl J Med.; 1992;327: 1041–4.

[21] Landgren BM, Johannisson, Aedo AR, Kumar A, Yong-en Shi. The effects of LNG administered in large doses at different stages of the cycle on ovarian function and endometrial morphology. Contraception 1989;39:275–89.

[22] Pakarinen P, Luukkainen T, Laine H, Lahteenmaki P. The effect of local intrauterine LNG administration on endometrial thickness and uterine blood circulation. Hum Reprod 1995;10:2390–4.

[23] Mandelin E, Koistinen H, Koistinen R, Affandi B, Seppala M. LNG releasing intrauterine device wearing women express contraceptive glycodelin-A in endometrium during midcycle: another contraceptive mechanism? Hum Reprod 1997;12:2671–5.

[24] Peek MJ, Markham R, Fraser IS. The effects of natural and synthetic sex steroids on human endometrial cell proliferation. Hum Reprod 1995;10:2238–43.

[25] Odeblad E. Biophysical techniques of assessing cervical mucus and microstructure of cervical epithelium. In: Elstein M, Moghissi KS, Broth R, editors. Cervical Mucus and Human Reproduction. Copenhagen: Scriptor, 1972. p. 58–74.

[26] Daunter B, Chantler EN, Elstein M. Scanning electrone microscopy of cervical mucus: normal menstrual cycle and pregnancy. Br J Obstet Gynaecol 1976;83:738–43.

[27] Moghissi KS, Syner FN, McBride LC. Contraceptive mechanism of microdose norethindrone. Obstet Gynecol 1973;41:585–90.

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Feasibility of administering mifepristone as a once a month contraceptive pill

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Many women find the idea of a once-a-month contraceptive pill an attractive concept. Mifepristone has been shown to be effective as a contraceptive if administered in the early luteal phase. We tested the contraceptive efficacy of 200 mg of mifepristone on day luteinizing hormone (LH) + 2 in a group of 32 women who used a fertility monitor to identify the LH surge. We also recruited a control group, comprising 20 women who were trying to conceive. In this group, 12 women conceived during a total of 50 control cycles (probability of pregnancy 0.25–0.32). Women in the treatment group contributed to a total of 178 cycles and there were two pregnancies (probability of pregnancy 0.01). An LH surge was not detected in 34 cycles (19.1%). In 20 cycles (11.2%) this was due to imperfect use while 14 were monitor method failures (7.9%). Treatment with mifepristone in the early luteal phase did not disrupt the cycle length but women reported slight vaginal bleeding in 15% of the cycles. The combination of a home-use fertility monitor with once-a-month administration of mifepristone (especially if mifepristone is administered at the early luteal phase) is an acceptable contraceptive option with minimal side effects. Unfortunately, it is difficult to envisage how an easier way of defining the correct timing, which required less compliance, could be devised.

Key words: contraceptive/home use fertility monitor/LH surge/Mifepristone/once-a-month pill

Introduction

Hormonal contraception is used by almost 100 million women world-wide. However, many women are deterred from using it because of perceived risks to health such as breast cancer or side effects such as weight gain. Most of the risks and the side effects are the results of prolonged exposure to steroids and many women, in a variety of cultural settings, find the idea of a pill which they need take only once each month, an attractive concept (Rimmer *et al.*, 1992; Glasier *et al.*, 1999).

Progesterone is essential for the establishment and maintenance of human pregnancy. The anti-progesterone mifepristone is a synthetic 19-norsteroid, which acts by blocking the action of progesterone at the receptor level (Spitz and Bardin, 1993), and thus, has multiple potential anti-fertility actions. When administered in the early luteal phase mifepristone retards endometrial development, without disturbing the timing of menses (Swahn *et al.*, 1988; Berthois *et al.*, 1991; Maentausta *et al.*, 1993). It also alters uterine contractility to a pattern more usually seen in the late luteal phase (Gemzell-Danielsson *et al.*, 1990). In 1993 Gemzell-Danielsson and colleagues conducted a pilot study in which a single dose of 200 mg of mifepristone was given in the early luteal phase [2 days following the surge of the lutenizing hormone (LH) in urine]. Out of 124 cycles in which coitus took place during the fertile period, only one pregnancy was observed (Gemzell-Danielsson *et al.*, 1993). There was no disruption of the timing of the subsequent menstrual bleed, although in 35% of the cycles slight vaginal bleeding was reported 2–3 days after treatment.

The main problem in developing a once-a-month contraceptive is finding a means that, both reliably and easily, identifies the start of the LH surge. Gemzell-Danielsson tried to solve this problem by using the LH sticks for home urine testing (Ovu-quick; Organon). In their study 12 out of 169 cycles were deemed to be anovulatory. However, it is not possible to determine if the LH surge truly was absent, or if the method failed to detect a surge. The woman may have read the test result wrongly or even failed to perform a test on the appropriate day.

Unipath (Bedford, UK) have developed a technology that can be used in the home to monitor changes in urinary hormones. This system comprises disposable test sticks and a hand held monitor, which together are used to detect changes in the levels of oestrone-3-glucuronide (E3G), a urinary metabolite of oestradiol, and LH, to indicate the potentially fertile days leading up to ovulation. The time from the first significant rise of LH in the urine to ovulation is reported to be around 24–48 h (Collins, 1996). The monitor thus should provide a convenient method of identifying the early luteal phase. Summary data for up to six consecutive cycles can be stored in the monitor memory and these data can be retrieved.

We investigated the contraceptive efficacy of 200 mg of mifepristone on day LH + 2 in a group of women who used this monitor to identify the LH surge.

Materials and methods

This was a single centre study in healthy female volunteers, approved by the Lothian Research Ethics Committee. All subjects gave written informed consent to participation. Fifty-two sexually active women, with regular (25–32 day) menstrual cycles were recruited from a large Family Planning Clinic in Edinburgh. If the women had a significant medical condition or if they or their partners had a history of fertility problems, they were excluded from the study.

Treatment group

Thirty-two women were recruited to the treatment group. None had been taking hormonal preparations within the 2 months prior to the start of the study and all had had at least two spontaneous menstrual periods since stopping hormonal contraception. All women underwent screening at the time of recruitment including a routine physical and gynaecological examination. A venous blood sample was taken for full blood count, serum biochemistry and liver function. The study started on day 1 of the menstrual period following screening, and lasted for up to seven consecutive menstrual cycles in which subjects took 200 mg mifepristone once per month.

Control group

The control group consisted of 20 healthy women with regular menstrual cycles who were trying to become pregnant (for less than 6 months prior to the enrolment in to the study) and hence, were not using contraception. They were provided with a monitor, which they used according to the manufacturer's instructions. Women were advised that their chance of conception would be higher if they were to have sexual intercourse during the fertile period, identified by the monitor. The controls took part in the study until pregnancy occurred or for a maximum of six cycles if they did not conceive.

Procedure

All subjects and controls were provided with a home use hormone monitoring system (Unipath, Bedford, UK). The system comprises a hand-held monitor and disposable dual-assay urine test sticks, and is used to simultaneously detect LH and E3G levels in early morning urine. The monitor optically measures the intensity of the lines that form on the test sticks after sampling, and the system will delineate three levels of fertility (Low, High and Peak Fertility) according to the optical signal changes detected. Low fertility will be displayed from day 1 of the cycle, until the hormone levels rise above the baseline levels. A change from low to high fertility is triggered by detection of elevated E3G levels, to concentrations typically between 20 and 30 ng/ml. The change from high to peak fertility is triggered by the detection of an LH surge, typically with a concentration >30 IU/l.

Peak fertility is displayed on the day of the LH surge and on the following day. Subsequently high fertility is displayed for 1 day prior to a return to low fertility. At the start of each menses, the subjects pressed the 'm' button on their monitor to initiate that cycle of use, at a time suitable for testing the first urine of the day. For the rest of the month, the subjects were required to consult the monitor display each morning (3 h either side of the time when 'm' button was set) to determine whether they needed to perform a test that day. Beyond this 6 h time window the monitor would not accept a test. The system requests one test every day for up to a total of 10 or 20 tests, depending on the length of the woman's cycle, and the timing of her LH surge. Embedded software within the monitor collects and analyses data from each cycle to identify and display fertility status to the user, and stores data for several months.

Mifepristone (Laboratories Exelgyn, Paris, France) was taken 2 days after the day of the first day of peak fertility (LH surge). With each cycle, subjects followed the same protocol, and were reviewed by the investigator monthly, on day LH + 2. Just before taking the 200 mg tablet of mifepristone, a venous blood sample was taken, and later assayed for progesterone. At the beginning of the study, if the LH surge was not identified by day 21 of the cycle, the subject was instructed to continue testing, but mifepristone was not given in that cycle. The subject was also advised to use barrier contraception from day 21 until the onset of the next menses. After the second pregnancy (which occurred due to a failure in detecting an LH surge), we changed this practice. We calculated the estimated day of LH surge for each month based on information from the previous cycles. If the women did not detect an LH surge either within 3 days after the anticipated day of LH surge or by day 19, a blood sample was taken for rapid serum progesterone assay. If the progesterone level was >5nmol/l and if the woman was at risk of pregnancy, mifepristone was administered.

All subjects and controls kept a menstrual record card, recording all vaginal bleeding experienced during the study and the days on which they had sexual intercourse. Subjects also marked the first day of the peak fertility as identified by the monitor and the day of taking the study medication.

If menstruation was overdue by more than one week the investigator performed a pregnancy test. Provided this was negative, the subject continued in the study and the next cycle was deemed to start with the onset of menses. Since the effect of mifepristone taken in very early pregnancy is unknown, and teratogenic effects could not be ruled out, women who would not consider terminating any pregnancy were not recruited to the treatment group.

At the end of the study, the subjects attended for a final visit, when a routine physical and gynaecological examination was performed. Full blood count, serum biochemistry and liver function were reassessed.

The following definitions were created for the purpose of the study.

Imperfect use: was defined as failure to detect an LH surge through performing the test incorrectly (e.g. dipping a test stick in urine 30 or more min before it being read by the monitor), or failing to perform tests as requested by the monitor.

Monitor method failures: were defined as failure to detect an LH surge despite performing all tests as requested.

High fertile days: days preceding the urinary LH surge as indicated by the monitor to be potentially fertile.

Peak fertile days: The first day of a significant rise in urinary LH detected by the monitor, and the following day.

The fertile period: of the cycle was defined as 3 days before until 2 days after the urinary LH surge (LH–3 to LH+2).

Exposure cycles: were cycles in which women reported having sexual intercourse at least once during the fertile period.

Statistical analysis

Cycle lengths and serum progesterone concentrations were compared by two-sample *t*-tests. Confidence limits for efficacy were derived from confidence limits for relative risk calculated by the Greenland and Robins method (Greenland and Robins, 1985).

Results

Table I shows the demographic characteristics of the women who took part in the study.

Table I. Demographic data.

	Treatment group (n = 32)	Control group (n = 20)
Age		
Range	18–39	26–40
Mean (± SD)	30 (± 5.4)	32.9 (± 4.5)
BMI		
Range	19–38	21–29
Mean (± SD)	23.6 (± 4.3)	23.8 (± 2.7)
Smokers (%)	7 (21.9)	1 (5)
Non-smokers (%)	21 (65.6)	16 (80)
Ex-smokers (%)	4 (12.5)	3 (15)
Previous pregnancies		
1+ (%)	19 (59.4)	14 (70)
Never been pregnant (%)	13 (40.6)	6 (30)
Ever abortion (%)	15 (46.9)	5 (25)
Married/Co-habiting (%)	28 (87.5)	20 (100)
Single (with a regular boy friend) (%)	4 (12.5)	0 (0)

The women in treatment group were slightly younger (mean age 30 years) than those in the control group (mean age 32.9 years). Otherwise there were no differences between subjects and controls.

The probability of pregnancy in the control group

Twenty women were recruited to the control group and three withdrew before completing the study. Two withdrew from the study as they found using the system 'too stressful' and one withdrew because she no longer wished to plan a pregnancy. Data were collected from 50 control cycles during which 12 pregnancies occurred. Average frequency of intercourse was 1.7 episodes per week in the 39 control cycles in which the women kept a record of their sexual activity. In 37 cycles women had intercourse at least once during the fertile period (FP). In two cycles intercourse did not occur during the FP, while in 11 cycles the exposure status was unknown, as women failed to keep a record of sexual activity. Eight pregnancies occurred in the first exposure cycle.

If we assume that all 11 cycles from which information on sexual activity was lacking were exposure cycles, the probability of pregnancy was 0.25. However if those cycles were all non-exposure cycles, the probability of conception would be 0.32. Therefore among the control group the overall probability of pregnancy if sexual intercourse took place at least once during the fertile period lies between 0.25–0.32.

Contraceptive efficacy of the method

Thirty-two volunteers were treated with a single dose of 200 mg of mifepristone administered in the luteal phase of the cycle as their sole method of contraception between one and seven cycles. They contributed a total of 178 cycles, and in 167 cycles mifepristone was administered. Eight women withdrew from the study before completion; two women moved out of the area, three ended their relationship, two conceived during the study and one lost confidence in the method.

Two clinical pregnancies occurred in the 178 cycles studied. The first pregnancy was a true treatment failure, which occurred in a woman (para 1) who took mifepristone on day 14 (LH + 2) of her first treatment cycle. She opted for a surgical termination of pregnancy, which was performed at 8 weeks of gestation (confirmed by ultrasound scanning). In the second woman (para 3), an LH surge was not identified in her third study cycle hence she did not receive treatment with mifepristone, menses did not occur and on day 37 after her last menstrual period an ectopic pregnancy was diagnosed and treated surgically. In a third woman a biochemical pregnancy was diagnosed (serum βHCG only rising to 34 IU/l), which was spontaneously and completely aborted by day 34 of the third study cycle after taking mifepristone on day 14 (LH + 2). This woman continued in the study and completed six treatment cycles.

The mean frequency of sexual intercourse was 1.8 episodes per week in 167 treatment cycles in which sexual activity was recorded. If we assume the probability of pregnancy in the treatment group is similar to the control group (0.25–0.32), the expected number of clinical pregnancies during the 178 cycles (in which 140 were exposure cycles) studied should be between 35–48.3. The observed number was two. Therefore, the efficacy of the method is 94.3% (95% confidence interval 75.4–98.7) – 95.9% (95% CI 82.5–99.0).

When calculating the efficacy of the method, we excluded the 29 cycles during which women were not exposed to a risk of pregnancy, and the three cycles in which mifepristone was taken in the follicular phase.

Contraceptive efficacy of luteal phase administration of mifepristone

In 145 cycles in which mifepristone was taken in the early luteal phase (within 2 days of the urinary LH surge) 117 were exposure cycles (Table II). Exposure status was unknown in eight cycles and in 20 cycles women were not at risk of pregnancy. In the 117 exposure cycles, there was only one clinical pregnancy.

In 19 (10.7%) cycles, no LH surge was declared by the monitor but mifepristone was given as coitus had taken place during the fertile period of the cycle (calculated according to the usual cycle length and usual day of LH surge). Occurrence of ovulation was confirmed by serum progesterone of >5 nmol/l in all 19 cycles and treatment was administered prior to day 21 of the cycle in each case [between day 13–21 of the cycle, mean 16.9 (SD ± 2.1) days]. There were no pregnancies in these cycles.

If the probability of pregnancy in all exposure cycles in the study is 0.25–0.32 (the same as that of the control group), between 34–46 clinical pregnancies would be expected in the 136 ovulatory cycles in which mifepristone was taken in the luteal phase. The observed number was one. Hence, the contraceptive efficacy of luteal phase mifepristone is between 97.1% (95% CI 78.00–99.6) – 97.8% (95% CI 83.9–99.7).

Performance of the home use hormone monitor

In 140 treatment cycles an LH surge was identified by the monitor, which equates to 90.9% LH surge detection when calculated for perfect use cycles; and 80.5% when imperfect use cycles are also included in the total. In 127 cycles this

Table II. Treatment cycle details.

	Total no. cycles	Exposure cycles	Unknown exposure	No Exposure
Mifepristone administered	167	136	8	23
In follicular phase	3	0	0	3
In luteal phase	164	136	8	20
Early luteal phase	145	117	8	20
LH + 2	127	100	7	20
LH + 1	17	16	1	0
LH + 0	1	1	0	0
In luteal phase (unknown LH status)	19	19	0	0
Mifepristone not given	11	5[a]	0	6[b]
Total	178	140	8	29

[a]LH surge missed, at risk of pregnancy but after day 21.
[b]Anovulatory cycle $n = 1$, LH surge missed and no risk of pregnancy $n = 5$).

was confirmed by a subsequent rise in serum progesterone of >5 nmol/l in the early luteal phase. This information was not available from nine cycles (blood samples lost or not collected). In the remaining four cycles serum progesterone was between 2–5 nmol/l, 1 or 2 days following the urinary LH surge as detected by the monitor. This may have been due to an early detection of the first significant rise in urinary LH. None of these five cycles were prolonged after taking mifepristone, hence it is unlikely that they were anovulatory.

There was a total of 38 (21.3%) cycles in which an LH surge was not detected. Among them, one (0.6%) was an anovulatory cycle, defined by serum progesterone not rising above 5 nmol/l in the mid-luteal phase. In three (1.7%) other cycles we administered mifepristone on day 19, before the monitor had identified an LH surge. Serum levels of progesterone (taken on the day of administering mifepristone) confirmed that in these cycles mifepristone was administered in the follicular phase. All three cycles were prolonged (43–52 days).

In the remaining 34 cycles an LH surge probably occurred (as suggested by a rise in serum progesterone of >5 nmol/l) but was not identified by the monitor. Fourteen were missed due to monitor method failure (7.9%) and 20 were a consequence of imperfect use of the system (11.2%).

Cycle length

Mifepristone when given in early luteal phase did not significantly affect the cycle length ($P = 0.35$). The mean of the usual cycle length was 28.3 days (SD ± 1.3) and during the treatment cycles it was 28.0 days (SD ± 1.9).

Side effects

Women kept a record of vaginal bleeding in 139 out of the total 144 cycles where mifepristone was taken on LH + 2. Mifepristone induced vaginal bleeding within 72 h in 21 cycles (15%). In a further 19 cycles, our volunteers took mifepristone in the luteal phase but the LH status was not known. In 17 of those cycles (>89%), mifepristone induced a vaginal bleed.

Serum progesterone values in blood samples taken just prior to mifepristone administration were available for 136 cycles. The mean serum progesterone value was significantly ($P < 0.0001$) higher in those cycles where mifepristone induced bleeding when compared to the mean value for the cycles without bleeding [21.72 (SD ± 9.04) nmol/l versus 13.33 (SD ± 6.23) nmol/l].

Two women spontaneously reported improvement of their pre-menstrual symptoms during cycles in which mifepristone was administered, while one reported worsening. In one woman hepatic alanine aminotransferase (ALT) was elevated at 103 IU/l (normal range 10–40 IU/l) at the end of the study but returned to normal within 2 months. One woman complained of diarrhoea 12 h post mifepristone in one cycle, three reported menstrual cramping within 72 h of taking mifepristone; two women reported a reduction in menstrual blood loss.

Discussion

A single dose of 200 mg of mifepristone administered once a month is an effective contraceptive method with an overall efficacy of 95% increasing to 97% if administered at the correct time (i.e. the early luteal phase). Thus our results are in agreement with the findings of a previous study (Gemzell-Danielsson *et al.*, 1993).

One criticism of previous work in this field has been the lack of a suitable control group for the subjects studied. Unlike the Gemzell-Danielsson study, we were able to compare the results with a contemporaneous control group using the same methodology in the same cultural setting. In this control group, if sexual intercourse took place on a fertile day the probability of a pregnancy was 0.25–0.32. The calculated probability of pregnancy in a cohort of couples monitored during a study of natural family planning (WHO, 1983) was 0.486 if intercourse took place 3 days prior to and a day after the peak day of mucus discharge. The difference in the probability of pregnancy between our study and a variety of other published series (Table III) may be explained by the fact that we have extended our definition of the fertile period to 6 days (3 days prior to the urinary LH surge until 2 days after). Other authors (Wilcox *et al.*, 1995) have calculated that the likelihood of conceiving during an ovulatory cycle to be 0.37 (95% confidence interval 0.31–0.48) if daily sexual intercourse took place during a 6 day fertile period (four days before and a day after ovulation). The lower frequency of intercourse in

Table III. Probability of clinical pregnancy.

	No. of exposure cycles	No. of pregnancies	Probability of pregnancy
Wilcox *et al.*, 1995[b]	129	34	0.26
Our control group[b]	37–48	12	0.25–0.32
Our treatment group[b] (monitor + mifepristone)	140–151	2	0.01
Our treatment group[b] (mifepristone in luteal phase)	136–143	1	0.007
Gemzell-Danielsson *et al.*, 1990[a]	124	1	0.008
WHO study[a]	72	35	0.48

[a]The length of the fertile period defined as 4 days.
[b]The length of the fertile period defined as 6 days.

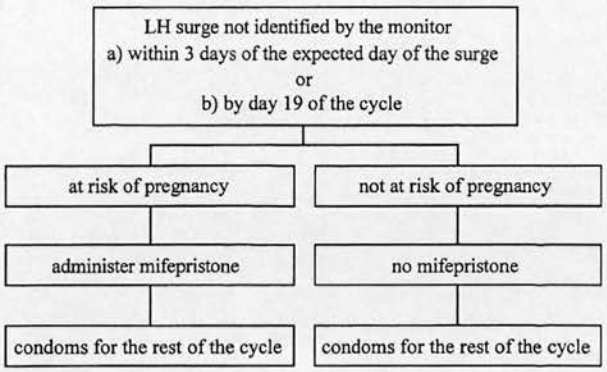

Figure 1. Algorithm for administering mifepristone when the LH surge is not identified

our group (untimed intercourse averaging 1.7 per week) may also explain the lower probability of pregnancy.

The limiting factor in this once-a-month approach to administering anti-progesterone is the accurate detection of the LH surge. Clearly, the failure to detect accurately the LH surge has a big impact on the overall effectiveness of the method. Using laboratory assay of LH in blood or urine to identify ovulation is neither practical nor convenient for long term use in the general population. The monitor provided us with an opportunity to overcome these problems. Gemzell-Danielsson *et al.* (1993) reported 49% accuracy using home LH detection sticks (Gemzell-Danielsson *et al.*, 1993). Although the monitor performed better (over 80.5% accuracy), both of these methods remain below the required standard. We studied 32 women over a total of 178 cycles. Imperfect use of the system accounted for failure to identify an LH surge in 11.8% cycles while 7.9% were due to monitor method failure. Compliance difficulties are associated with all contraceptives and non-compliance in ~12% of cycles is probably no worse than with any other method which demands action from the user, for example, compliance rates reported from oral contraceptive pill users range from 3.4–100% (Wheble *et al.*, 1981; Molloy *et al.*, 1985; Hamilton and Hoogland, 1989). Although our study population consisted of women who were motivated and committed and some of them already had experience in using natural family planning methods, they found the short, inflexible testing window set on day 1 of the cycle to be particularly demanding. This is inconsistent with couples using the monitor in order to get pregnant (Bonnar *et al.*, 1999). The prevalence of imperfect use is likely to rise in the general population compared with that typical of a research study.

During the course of the study we developed an algorithm (Figure 1) for the administration of mifepristone if an LH surge was not identified. In 19 exposure cycles (out of 28 cycles in which an LH surge was not identified) mifepristone was administered using this algorithm and there were no pregnancies. Given that the methods available to be used in real life to time the administration of mifepristone cannot be 100% accurate, such an algorithm will be essential to deal with a missed LH surge.

In our study, mis-timed administration of mifepristone led to predictable effects. When administered during the proliferative phase of the menstrual cycle, mifepristone inhibited follicular development, and delayed the mid cycle LH surge, leading to a delay in ovulation and subsequent prolongation of the menstrual cycle (Liu *et al.*, 1987; Luukkainen *et al.*, 1988; Swahn *et al.*, 1988). Ovulation may occur later in that cycle, leaving women at risk of conception. In our study, when administered in the late follicular phase (in error) in three cycles, mifepristone prolonged the cycle length (43–52 days). The women were advised to use condoms for the remainder of that cycle and none of the three cycles resulted in pregnancy.

Administration of mifepristone in the mid or late luteal phase induces a bleed within a few days of treatment, which may or may not be followed by a second bleed at the time of expected menstruation (Shoupe *et al.*, 1987; Swahn *et al.*, 1988). In our study, in 17 out of the 19 cycles where mifepristone was taken after ovulation (the LH status unavailable and probably later than on LH + 2), intermenstrual vaginal bleeding occurred (89.5%). Moreover, there was an increased risk of bleeding seen in those women who may have taken mifepristone slightly later in the LH + 2 window. The mean serum progesterone concentration was significantly higher in those women who had bleeding after taking mifepristone within LH + 2, when compared with those who did not. The higher serum progesterone value in some on LH + 2, could be due to a delayed identification of the first significant rise in urinary LH, or because of a more rapid increase in serum progesterone due to early ovulation. Nevertheless, in our group of women, in all cycles where mifepristone induced a vaginal bleed, a second bleed occurred at the time of the expected menses. Therefore, while the bleeding may have been inconvenient, it did not jeopardise efficacy or continued use of the method. There was less inter-menstrual bleeding (15% of the cycles) reported in our study when mifepristone was taken within LH + 2, less than half of that reported by Gemzell-Danielsson *et al.* (32%) (Gemzell-Danielsson *et al.*, 1993). This is possibly due to the fact that the majority of women in our study received mifepristone at the correct time. In their study, in 51% of the cycles, mifepristone was taken between 3 and 5 days after the LH surge.

In conclusion, the use of the combination of home use

fertility monitor with once-a-month administration of mifepristone (especially if mifepristone is administered at the early luteal phase) is an attractive contraceptive option with minimal side effects. However, to be an effective contraceptive method, the women have to be committed to using a device, which identifies the LH surge, in order that the pill can be taken at the correct time in the cycle. Whilst this regimen may be acceptable to motivated women, it may be regarded as too complicated for others to adopt on a routine basis. There was evidence of such non-compliance in this study, with 11.2% of LH surges being missed as a consequence of imperfect use of the monitor. Unfortunately, it is difficult to envisage how an easier way of defining the correct timing, which obligated less compliance, could be devised.

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References

Berthois, Y., Salat-Baroux, J., Cornet, D. *et al.* (1991) A multi-parametric analysis of endometrial oestrogen and progesterone receptors after post-ovulatory administration of mifepristone. *Fertil. Steril.*, **55**, 574–554.

Bonnar, J., Flynn, A., Freundl, G. *et al.* (1999) Personal hormone monitoring for contraception. *Brit. J. Fam. Plann.*, **24**, 128–134.

Collins, W.P. (1996) Indicators of potential fertility: scientific principles. In Bonner, J. (ed) *Natural Conception Through Personal Hormone Monitoring.* New York: The Parthenon Publishing Group, pp. 13–33.

Gemzell-Danielsson, K., Swahn, M.-L., and Bygdeman, M. (1990) Regulation of non-pregnant human myometrial contractility. Effects of anti-hormones. *Contraception*, **42**, 323–335.

Gemzell-Danielsson, K., Swahn, M.-L., Svalander, P. and Bygdeman, M. (1993) Early luteal phase treatment with mifepristone (RU 486) for fertility regulation. *Hum. Reprod.*, **8**, 870–873.

Glasier, A.F., Smith, K.B., Cheng, L. *et al.* (1999) An international study on the acceptability of a once-a-month pill. *Hum. Reprod.*, **14**, 3018–3022.

Greenland, S. and Robins, J.M. (1985) Estimation of a common effect parameter from sparse follow-up data. *Biometrics*, **41**, 55–68.

Hamilton, C.J.C.M. and Hoogland, H.J. (1989) Longitudinal ultrasonographic study of the ovarian suppressive activity of a low dose triphasic oral contraceptive during correct and incorrect pill intake. *Am. J. Obstet. Gynecol.*, **161**, 1159–1162.

Liu, J.H., Garzo, G., Morris, S. *et al.* (1987) Disruption of follicular maturation and delay of ovulation after administration of the antiprogesterone RU 486. *J. Clin. Endocrinol. Metab.*, **65**, 1135–1140.

Luukkainen, T., Heikinheimo, O., Haukkamaa, M., and Lahteenmaki, P. (1988) Inhibition of folliculogenesis and ovulation by the antiprogesterone RU 486. *Fertil. Steril.*, **49**, 961–963.

Maentausta, O., Svalander, P., Gemzell-Daniellsson, K. *et al.* (1993) The effects of an antiprogestin, mifepristone and antiestrogen tamoxifen on endometrial 17β-hydroxysteroid dehydrogenase and progestin and estrogen receptors during the luteal phase of the menstrual cycle: An immunohistochemical study. *J. Clin. Endocrinol. Metab.*, **77**, 913–918.

Molloy, B.G., Coulson, K.A., Lee, J.M. and Watters, J.K. (1985) 'Missed pill' conception: fact or fiction? *BMJ*, **290**, 1474–1475.

Rimmer, C., Horga, M., Cerar, V. *et al.* (1992) Do women want a once-a-month pill? *Hum. Reprod.*, **7**, 608–611.

Shoupe, D., Mishell, D.R., Lahteenmaki, P. *et al.* (1987) Effects of the antiprogesterone RU 486 in normal women. *Am. J. Obstet. Gynecol.*, **157**, 1415–1420.

Spitz, I.M. and Bardin, C.W. (1993) Clinical pharmacology of RU 486 – an antiprogestin and antiglucocorticoid. *Contraception*, **48**, 403 – 444.

Swahn, M.-L., Johannisson, E., Daniore, V. *et al.* (1988) The effect of RU 486 administered during the proliferative and secretory phase of the cycle on the bleeding pattern, hormonal parameters and the endometrium. *Hum. Reprod.*, **3**, 915–921.

Wheble, A.M., Street, P. and Wheble, S.M. (1981) Contraception: Failure in practice. *Br. J. Fam. Plann.*, **7**, 41–44.

WHO Task Force on Methods for the determination of the fertile period (1983) A prospective multicentre trial of the ovulation method of natural family planing. III. Characteristics of the menstrual cycle and of the fertile phase. *Fertil. Steril.*, **40**, 773–778.

Wilcox, A.J., Weinberg, C.R. and Baird, D.D. (1995) Timing of sexual intercourse in relation to ovulation: effects on the probability of pregnancy and sex of the baby. *NEJM.*, **333**, 1517–1521.

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Noncompliance among a group of women using a novel method of contraception

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Objective: To compare the incidence of noncompliance measured objectively by a home use fertility monitor with the traditional self-reported incidence of compliance in a study of a new method of contraception.

Design: Prospective cohort study.

Setting: A large family planning clinic in Edinburgh.

Patient(s): Thirty-two healthy women who took part in a trial assessing the efficacy of a novel method of contraception involving accurately timed administration of a single dose of mifepristone.

Intervention(s): Mifepristone was administered orally and a blood sample was collected on the same day.

Main Outcome Measure(s): Percentage of missed tests detected by the monitor against the self-reported percentage during the critical period.

Result(s): Women failed to perform 24.2% (95% confidence interval, 16.5–31.5) of the tests in the 162 cycles analyzed. They missed tests at an absolutely vital time for contraceptive efficacy in 42% of cycles according to the monitor while admitting to missing tests in 14.8%. Poor compliance was associated with younger women, those who discontinued the study before completion, and cycles in which women were not relying on the contraceptive method.

Conclusion(s): The use of microelectronic monitoring systems may improve our understanding of the extent of patient noncompliance, providing objective information that no other monitoring technique can produce. This understanding provides the opportunity to make the optimum use of potentially effective treatments while validating research evidence. (Fertil Steril® 2001;76:1196–1201. ©2001 by American Society for Reproductive Medicine.)

Key Words: Compliance, contraceptive research, home-use fertility monitor, mifepristone

Since the first woman on earth chose to contradict the instructions of her Provider in the Garden of Eden, expecting her descendants to comply perfectly with a contraceptive regimen may seem rather unrealistic. However, noncompliance with a particular contraceptive method is linked with an increased risk of unintended pregnancy (1). Conventionally, it has been assumed that the users of contraception are highly motivated because the consequence of noncompliance —pregnancy—is so obvious, and so significant. Studies involving organ transplant recipients have shown, however, that no consequence of poor compliance is severe enough—not even the rejection of a transplanted kidney—for all patients to reliably follow their prescribed regimen (2).

Poor compliance—in both clinical practice and research—is associated with a number of factors. Though several patient characteristics (e.g., age, education, socioeconomic background) (3–5), characteristics of the treatment regimen (e.g., frequency of dosing, side effects) (6–8), and outcome characteristics (e.g., treatment of incurable or terminal illnesses) (9) have been associated with nonadherent behavior, there are no reliable and universally applicable predictors of noncompliance. In addition, there is no gold-standard measurement for patient compliance (10).

Prevalence of noncompliance ranges from 0–96.6% in oral contraceptive pill users (11–13). Although extremely common, there is a dearth of information available on patient noncompliance with the use of different contraceptive methods. What data exist commonly come from self-reporting. When undetected, poor compliance can invalidate results of efficacy

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studies (14, 15), yet it is commonly overlooked in clinical research (16).

We observed behavior of 32 women who took part in a trial assessing the efficacy of a novel method of contraception (17). The women took 200 mg of mifepristone 2 days after the midcycle LH surge (measured in urine) as a once-a-month contraceptive pill. Mistimed administration of mifepristone can disrupt the menstrual cycle and can also leave the women at risk of conception. A home use fertility monitor that could store data on daily testing events was used to time the administration of mifepristone. Although it made minimal demands on the users, the method provided objective, long-term data on their routines. Because investigators saw participants once each cycle throughout the study, we were able to compare the results of this method with the traditional self-reported incidence of compliance.

MATERIALS AND METHODS

Data were collected during a study assessing the feasibility of administering mifepristone as a once-a-month contraceptive pill, and detailed study methodology has been reported elsewhere (17). Thirty-two sexually active women (age range, 18–39 years) were enrolled from a large family-planning clinic in Edinburgh. (The mean body mass index was 24 ± 4.3, and 66% were nonsmokers.) All subjects gave written informed consent to participate, and the Lothian Research Ethics Committee approved the study. Data were collected from a total of 178 cycles, with each subject contributing between one and eight cycles. It was not possible to retrieve compliance data from the monitors in 28 of the study cycles because of infrequent downloading from the monitor and lost or broken devices.

The study started on day 1 of the menstrual period after screening and lasted for up to eight consecutive menstrual cycles. The subjects were relying on once-a-month treatment with mifepristone as their sole method of contraception during 150 of the cycles analyzed. In addition, it was possible to retrieve data from a further 12 cycles during the study suspension period, during which women did not rely on our method for contraception but only used the monitor. During the cycles in which mifepristone was administered, the timing of mifepristone depended on detection of the LH surge, which in turn depended on compliance with daily urine testing.

Procedure

All subjects were provided with a home use fertility monitor (Unipath, Bedford, UK). The system comprises a hand-held monitor and disposable dual-assay urine test sticks and is used to detect simultaneously LH and estrone-3-glucuronide levels in early-morning urine. The system delineates three levels of fertility (low, high, and peak fertility) according to the optical signal changes detected. At the start of each menses, the subjects pressed the *m* button on their monitors to initiate that cycle of use at a time suitable for testing the first urine of the day.

For the rest of the month, the subjects were required to consult the monitor display each morning (3 hours on either side of the time when the *m* button was set) to determine whether they needed to perform a test that day. Without this 6-hour time window, the system would not accept a test. The monitor requests one test every day for up to a total of 10 or 20 tests, depending on the length of the woman's cycle and the timing of her LH surge. Embedded software within the monitor collects, analyzes, and stores data for several months.

The correct way to use the monitor (including the importance of the testing window) was demonstrated to all subjects at the time of recruitment, and written information was given. Subjects were advised to contact the investigator immediately if they were not able to perform a test during a critical period. The investigator was available by telephone 7 days a week.

In each cycle, subjects were reviewed by the investigator monthly, on day LH+2. A sample of venous blood was collected before taking the tablet of 200 mg mifepristone and was later used for the analysis of progesterone. The information collected by the monitor was later analyzed using special computer software. The estimated day of LH peak for each month was calculated based on information from all the previous cycles monitored. If an LH surge was not detected either within 3 days after the anticipated day of LH peak or by day 19 of the cycle, a blood sample was taken for rapid serum progesterone assay, and the information from the monitor was downloaded. Mifepristone was administered only if the woman was at risk of pregnancy (i.e., had been sexually active) and if the progesterone level was >5nmol/L.

All subjects kept a menstrual record card, recording all vaginal bleeding experienced during the study and the days on which they had sexual intercourse. They also marked the day of the LH surge as identified by the device and the day of taking the study medication.

The following definitions were created for the purpose of the study. The period of time between the calculated earliest day an LH peak was likely to occur (based on the usual cycle length and the day of the LH surge in previous cycles) and the day of the actual LH surge in each cycle was defined as the *critical period* for each patient. The *fertile period* of the cycle was defined as 3 days before until 2 days after the urinary LH surge (LH−3 to LH+2). *Exposure cycles* were cycles in which women reported having sexual intercourse at least once during the fertile period. *Noncompliance* was defined as a urine test that was requested by the monitor but missed.

Statistical Methods

Some of the compliance data were summarized for descriptive purposes using numbers of cycles or tests as de-

nominators. However, statistical inference was carried out on data aggregated to patient level, to take account of possible heterogeneity in behavior among patients that might have invalidated analyses using data from individual cycles or tests. Thus, percentages of tests missed were calculated for each patient, and these were tested for association with demographic data using Pearson correlations. Testing was done on data aggregated to patient level. Thus, for example, correlating each patient's age with the percentage compliance over all her cycles tested the association between age and compliance. Similarly, percentages of tests missed at different stages of the cycle, and when on and off the treatment regime, were compared by paired *t*-tests. A two-sample *t*-test was also used to compare the percentage of tests missed in patients who dropped out and those who did not.

RESULTS

One hundred sixty-two cycles were analyzed, of which 150 were study cycles. Data collected during the 12 cycles during which subjects were using a barrier method throughout the cycle and did not receive mifepristone were analyzed separately.

In total, 2,013 tests were requested by the monitor during the 162 cycles analyzed (12.4 tests per cycle, 95% confidence interval [CI], 11.8–13.0), and 494 were missed (24.2%; 95% CI, 16.5–31.5). On average, three tests (95% CI, 2.4–3.6) per cycle were missed.

Compliance During the Intention-to-Treat Cycles

During the 150 cycles in which women were relying on the study method as their only contraception, a total of 1,816 tests was requested by the monitor, and 411 tests were missed (22.6%; 95% CI, 15.2–30.1).

Compliance Before and After Identifying the Peak

In the days before the women knew an LH surge had occurred, 23% of the requested tests were missed (95% CI, 16–30; 260 of 1,160). Women were not more likely to miss tests before the LH surge than after it ($t = 0.57$, NS).

Concordance of Monitor Data With the Self-Reported Data

In 68 cycles (42%), women failed to test at all on a day of the cycle, which was critical to the accurate detection of an LH surge. In 27 of these cycles, the monitor did not detect an LH peak. In the remaining 41 cycles, despite critical tests being missed, the monitor did detect an LH peak. Women admitted to not performing tests in 24 cycles (14.8%). In the other 44 cycles, women did not report missed tests and only admitted to it once the investigator showed the downloaded monitor data to them.

Some women actively fabricated the information that they reported to the investigator. In one of these cycles, when contacted, a woman declared detecting an LH peak on a day (day 13 of the cycle) when she had not performed a test at all. Another woman performed the tests correctly and identified an LH peak on day 13 but forgot to inform the investigator and failed to obtain the mifepristone tablet as per protocol. When contacted on day 19 of the same cycle, she claimed that the monitor had not detected an LH peak.

Compliance During the Critical Days

Noncompliance with urine testing as monitored by the system was significantly lower during the critical period (15.6%; 95% CI, 9.5–21.7) when compared with during the noncritical days (27.5%; 95% CI, 17.9–37; paired *t*-test, $t = 3.64$, $P<.01$). The self-reported percentage of missed tests during the critical period was 2.7% (95% CI, 1.0–4.3), which was significantly lower than that detected by the monitor ($t = 4.48$, $P<.001$).

Compliance During the Study Suspension Interval

After a pregnancy that occurred because of a failure in detecting a LH peak, the study was suspended for a month. However, some women continued to use the monitor only, while using a barrier method for contraception. Therefore, it was possible to retrieve data from the monitors for 12 cycles in which the women were not using our method as their contraceptive.

In six of these 12 cycles, the monitor was not able to identify an LH peak because of its imperfect use. During this period, a significantly high percentage of tests (41.2%; 95% CI, 22.4–60.0) were missed when compared with the study cycles (22.6%; 95% CI, 15.2–30.1; $t = 2.9$, $P=.015$).

Compliance and Sexual Activity

There was no correlation between the frequency of sexual intercourse per cycle and the number of missed tests per cycle (correlation coefficient was 0.01). The percentage of missed tests were compared between the exposure and nonexposure cycles in 12 women who had at least one exposed and one unexposed cycle. There was no significant difference (paired *t*-test, $t = 0.06$, NS; 95% C.I for differences between exposed and unexposed, -8.3 to $+7.8$) in compliance during exposed and nonexposed cycles.

Demographic Features and Compliance

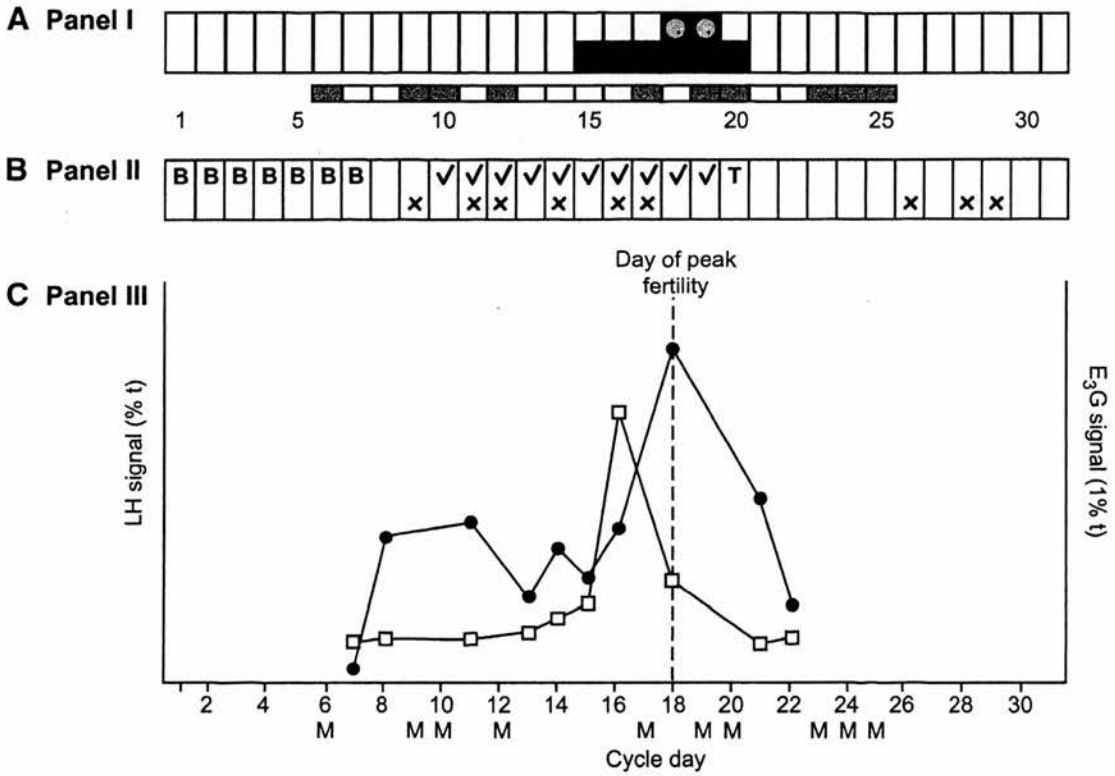
Age was negatively correlated with the percentage of missed tests—younger women missed more tests ($r = -0.36$, $P<.05$). There was no apparent relationship between compliance with the method and the number of pregnancies, number of previous abortions, or number of living children.

Compliance Among Dropouts

We analyzed cycle data from the seven women who discontinued the study before completion. They missed a significantly higher percentage of tests (44.4%) when compared with the other 25 women who completed the study

FIGURE 1

Information collected from the monitor and the diary card from a woman with a 31-day cycle in which the LH surge was identified. Mifepristone was administered on day 20 (LH+2). (**A**), Information downloaded from the monitor. Level of fertility displayed to the woman on each day of the cycle: open block, low fertility; partially closed block, high fertility; closed block with circle, peak fertility. Data on testing events appears as a narrower bar underneath: open block, test performed; closed block, test missed. (**B**), Data recorded on the diary card by the woman (self-report). B, days of vaginal bleeding; check mark, tests done during the critical period; X, days in which sexual intercourse occurred; T, day when mifepristone was taken. (**C**), Downloaded information on signal levels of LH and estrone-3-glucuronide (E3G) levels. Closed circle, LH; open square, E3G; M, missed tests. Note that on several days (comparing panels **A** and **B**), the woman reported performing the test (check mark) when the monitor showed that she did not (M).



Hapangama. Poor compliance in contraceptive research. Fertil Steril 2001.

(17.4%; 95% CI of the differences between the two percentages, 10.6–43.3; $t = 3.39$, $P=.002$). The dropouts missed tests on a critical day in 16 of the 22 cycles, although they admitted to missing tests in only four. Therefore, the self-reported incidence of noncompliance during the critical period was 18.1%, whereas the monitor-detected incidence was 72.7%.

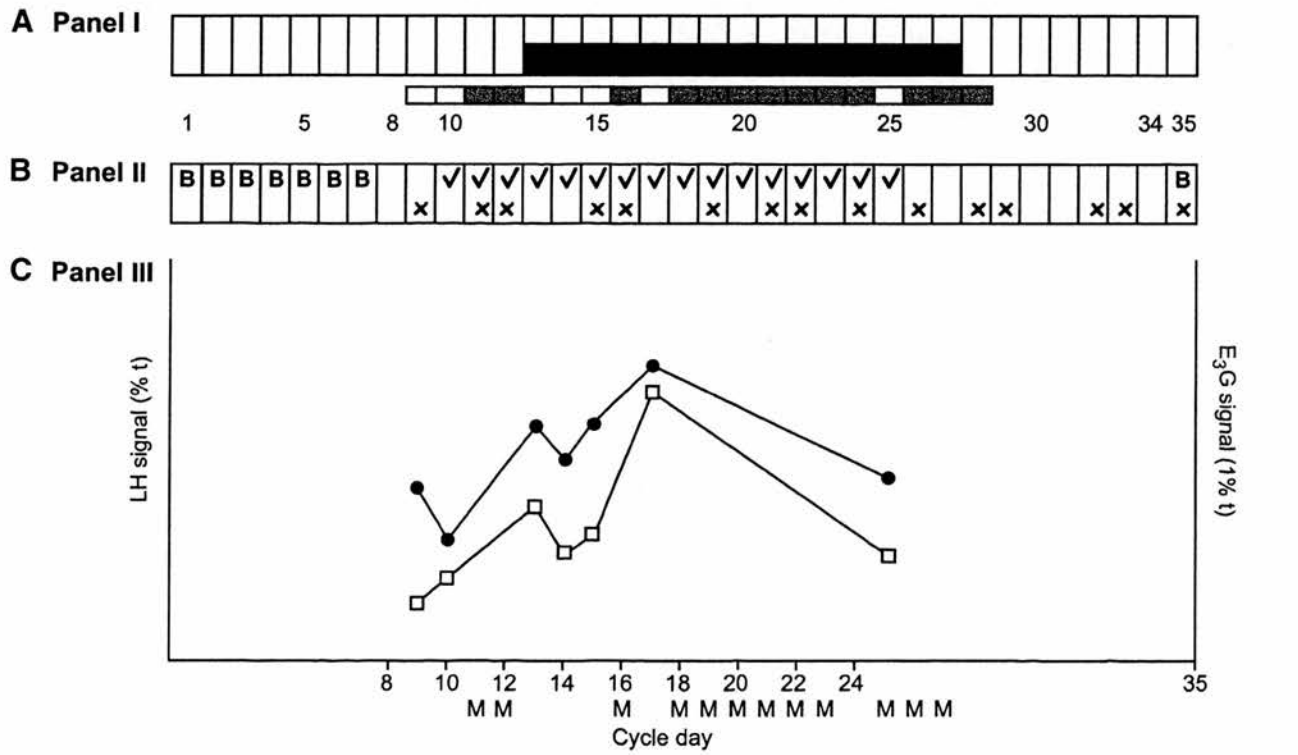
DISCUSSION

The home use fertility monitor offered us an important methodological advance in providing reliable data on the incidence and the magnitude of noncompliance with the contraceptive method studied and on information about exactly when the imperfect use occurred. We sought to achieve significantly high compliance rates with employing this monitoring system in a contraceptive regimen, which appeared to be acceptable to women (18, 19). Women were counseled at the start of the study regarding the importance of performing urine tests accurately to identify the time to administer mifepristone. They were aware that if mifepristone was not taken, they would be at risk of pregnancy.

Because the teratogenic effects of mifepristone are not known, women considering participation in the study were advised that if pregnancy occurred, they should consider termination. Despite this, the information collected from the monitor demonstrates that women failed to perform 24.2% of the tests in the 162 cycles analyzed. Moreover, in 42% of the cycles, women missed tests during a day that an LH surge was likely to have occurred, in other words, at an absolutely vital time for contraceptive efficacy.

FIGURE 2

Information collected from the monitor and the diary card from a 35-day cycle in the same woman as illustrated in Figure 1. As a consequence of missed tests, the monitor did not identify the LH surge, and mifepristone was not administered. (**A**), Information downloaded from the monitor. Level of fertility displayed to the woman on each day of the cycle: open block, low fertility; closed block, high fertility. Data on testing events appears as a narrower bar underneath: open block, test performed; closed block, test missed. (**B**), Data recorded on the diary card by the woman (self-report). B, days of vaginal bleeding; check mark, tests done during the critical period; X, days in which sexual intercourse occurred. (**C**), Downloaded information on signal levels of LH and estrone-3-glucuronide (E3G) levels. Closed circle, LH; open square, E3G; M, missed tests.



Hapangama. Poor compliance in contraceptive research. Fertil Steril 2001.

Although noncompliance is common, by inviting only a selected group of women who appeared to be motivated and committed, we sought to exclude those who might be poor compliers. Interestingly, the medical profession is reportedly poor in identifying noncompliers (20). Users of our method were reasonably young (mean age, 30 years) and were healthy. The serious outcome of noncompliance (pregnancy) was rare. Women may have found the daily requirement for urine testing, using the monitor on a long-term basis, to be complicated or inconvenient. Side effects of the method (although fewer than in most other methods available) may not have been acceptable to them, or they may not have had faith in the effectiveness of the method although this seems unlikely because they did not revert to condoms. Considering these odds, it is not difficult to infer why compliance problems were frequent.

Common sense suggests that with increasing experience, the women may trivialize the need for testing on days during which an LH surge was unlikely to have occurred. All women were made aware of the critical period for testing urine at the start of the study, and as expected, women missed fewer tests during this period. Incomplete understanding of how to be compliant with a regimen has been suggested as a common reason for poor compliance (21). Women in our study were given detailed verbal and written information on using the monitor at the start of the study. The importance of accurate use of the device according to manufacturer's guidelines was stressed at each monthly visit. Because there was no significant difference in the percentage of missed tests between the first study cycle and the last study cycle, it is difficult to support the suggestion that in the earlier cycles, women were less confident about how to use the method or more confident in their ability to guess the correct time for testing (22).

There was a highly significant difference between the patient self-reported percentage of missed tests (2.7%) and that detected by the monitor (15.6%) during the critical period. Figures 1 and 2 illustrate this discrepancy during two cycles contributed

by one woman. This discrepancy was even greater among the seven women who discontinued the study before completion and is consistent with results of previous studies that reported significant overestimation of compliant behavior with self-regulation (23). Patients tend to tell doctors what they think the doctors wish to hear. If they assume that doctors perceive patient nonadherence as a judgmental disappointment, they may feel guilty and fail to report noncompliance.

Although older women tended to miss fewer tests, the other demographic characteristics such as parity, previous abortions, number of living children, and educational background did not correlate with the number of missed tests. Neither did frequency of sexual activity or exposure to the risk of pregnancy correlate with the missed tests. However, because the information about sexual activity was only collected by self-reporting, this association should be accepted with caution.

Most investigators work on the assumption that a patient who complies with one aspect of a clinical protocol (e.g., attending the clinic as directed) also adheres to all other aspects of the study, though this might not always be the case. The monitor supplied data on daily testing of urine, which was only one part of the contraceptive method. We cannot infer from those data how well the women in our study complied with the rest of the protocol, such as recording daily events (sexual intercourse, vaginal bleeding). With no means of testing this, for validity of our results, we had to depend on our volunteers being truthful. Therefore, in the future, we have no other option but to work toward forming a true therapeutic alliance with our volunteers and to come to an agreement with our patients rather than to impose a prescription or a protocol upon them.

In conclusion, the use of microelectronics monitoring systems such as the home use fertility monitor may improve our understanding of the extent of the problem of patient noncompliance, providing precise objective information that no other monitoring technique can produce. This understanding will empower us as health care providers to adopt a no-fault approach to behavior relating to noncompliance and establish "a tailored consensual regimen" with the user that she is able to adhere to (24). This provides the opportunity to make the optimum use of potentially effective treatments and legitimate research evidence. Perhaps a small price to pay for such a return!

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References

1. Rosenberg M, Waugh MS, Long S. Unintended pregnancies and misuse and discontinuation of oral contraceptive. J Reprod Med 1995;40:355–60.
2. Rovelli M, Palmeri E, Bartus S, Hull D, Schweizer R. Non-compliance in organ transplant recipients. Transplant Proc 1989;21:833–4.
3. Meichenbaum D, Turk DC. Facilitating treatment adherence: a practitioner's guidebook. New York: Plenum Press, 1987.
4. Potter LS. Oral contraceptive compliance and its role in the effectiveness of the method. In: Cramer JA, Spilker B, eds. Patient compliance in medical practice and clinical trials. New York: Raven Press, 1991: 195–231.
5. Rapoff MA, Barnard MU. Compliance with paediatric medical regimens. In: Sackett DL, Haynes RB, eds. Compliance with therapeutic regimens. Baltimore, MD: John Hopkins University Press, 1986:73–98.
6. Puller T, Birtwell AJ, Wiles PG, Hay A, Feely MP. Use of a pharmacologic indicator to compare compliance with tablets prescribed to be taken once, twice, or three times daily. Clin Pharmacol Ther 1988;44: 540–4.
7. Rapoff MA. Compliance with treatment regimens for paediatrics rheumatic disease. Arthritis Care Res 1989;2:S40–7.
8. Cromer BA, Steinburg K, Gardner L, Thornton D, Shannon B. Psychological determinants of compliance in adolescents with iron deficiency. Am J Dis Child 1989;143:55–8.
9. Spilker B. Methods of assessing and improving patient compliance in clinical trials. In: Cramer JA, Spilker B, eds. Patient compliance in medical practice and clinical trials. New York: Raven Press, 1991:37–56.
10. Rudd P. In search of the gold standard for compliance measurement [editorial]. Arch Intern Med 1979;139:627–8.
11. Wheble AM, Street P, Wheble SM. Contraception: failure in practice. Br J Fam Plann 1981;7:41–4.
12. Hamilton CJCM, Hoogland HJ. Longitudinal ultrasonographic study of the ovarian suppressive activity of a low dose triphasic oral contraceptive during correct and incorrect pill intake. Am J Obstet Gynecol 1989;161:1159–62.
13. Molloy BG, Coulson KA, Lee JM, Watters JK. "Missed pill" conception: fact or fiction? Br Med J 1985;290:1474–5.
14. Probstfiels JL, Russell ML, Insull W, Yusuf S. Dropouts from a clinical trial, their recovery and characterization: a basis for dropout management and prevention. In: Shumaker SA, Schron EB, Ockene JK, eds. The handbook of health behaviour change. New York: Springer, 1990: 376–400.
15. Urquhart J. Non-compliance: the ultimate absorption barrier. In: Prescott LF, Nimmo WS, eds. Novel drug delivery and its therapeutic application. New York: Wiley, 1989:127–37.
16. Dahlstrom B, Eckernas S-A. Patient computers enhance compliance with completing questionnaires. In: Cramer JA, Spilker B, eds. Patient compliance in medical practice and clinical trials. New York: Raven Press, 1991:233–40.
17. Hapangama DK, Brown AH, Glasier AF, Baird DT. Feasibility of administering mifepristone as a once a month contraceptive pill. Hum Reprod 2001;16:1145–50.
18. Rimmer C, Horga M, Cerar V, Alder EM, Baird DT, Glasier A. Do women want a once-a-month pill? Hum Reprod 1992;7:608–11.
19. Glasier AF, Smith KB, Cheng L, Ho PC, van der Spuy Z, Baird DT. An international study on the acceptability of a once-a-month pill. Hum Reprod 1999;14:3018–22.
20. Gilbert JR, Evans CE, Haynes RB, Tugwell P. Predicting compliance with a regimen of digoxin therapy in family practice. Can Med Assoc J 1980;123:119–22.
21. Rosenburg MJ, Waugh MS, Meehan TE. Use and misuse of oral contraceptives; risk indicators for poor pill taking and discontinuation. Contraception 1995;51:283–8.
22. Lusher TF, Vetter H, Siegenthaler W, Vetter W. Compliance in hypertension: facts and concepts. J Hypertens 1985;3(Suppl 1):3–9.
23. Potter L, Okley D, de Leon-Wong E, Caneonar R. Measuring compliance among oral contraceptive users. Fam Plann Perspect 1996;28: 154–8.
24. Fink DL. Tailoring the consensual regimen. In: Sackett DL, Haynes RB, eds. Compliance with therapeutic regimens. Baltimore, MD: John Hopkins University Press, 1976:110–8.

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Mifepristone-Induced Vaginal Bleeding Is Associated with Increased Immunostaining for Cyclooxygenase-2 and Decrease in Prostaglandin Dehydrogenase in Luteal Phase Endometrium

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The mechanism of mifepristone-induced vaginal bleeding and endometrial shedding was investigated in 13 women who took 200 mg mifepristone in the midluteal phase on d 8 after the onset of the urinary LH surge (LH+8). Endometrial biopsies were collected, 6–24 h after mifepristone (group 1, n = 7) or 36–48 h after mifepristone (group 2, n = 6), and compared with those from a control group in the midluteal phase (n = 7). All women reported vaginal bleeding commencing 36–48 h after taking mifepristone. Treatment with mifepristone significantly reduced serum progesterone levels in all women, when compared with the controls (13.2 nM *vs.* 34.8 nM, $P = 0.001$). After mifepristone, a significant increase in cyclooxygenase-2 immunoreactivity was apparent at 36–48 h ($P = 0.0018$), whereas prostaglandin 15 dehydrogenase enzyme-positive immunostaining declined, to be virtually absent by 36–48 h in both glands and in stroma ($P < 0.05$). There was no change in intensity or distribution of staining for steroid receptors after mifepristone. The changes in immunostaining for cyclooxygenase-2 and prostaglandin 15 dehydrogenase strongly support the hypothesis that an increase in the local concentration of prostaglandins in the endometrium is involved in the mechanism of bleeding induced by mifepristone in the luteal phase. (*J Clin Endocrinol Metab* 87: 5229–5234, 2002)

HUMAN ENDOMETRIUM is a target organ for the ovarian steroid hormones estradiol and progesterone. One of the fundamental roles of progesterone is the differentiation of an estrogen-primed endometrium (1). The endometrial receptivity that permits successful implantation depends on timed and regulated synthesis and secretion of a specific set of progesterone-induced proteins in an estrogen-primed endometrium. If a pregnancy fails to occur, the corpus luteum regresses, with a subsequent fall in progesterone levels. There is now compelling evidence that a period of exposure of the estrogen-primed endometrium to progesterone followed by a withdrawal of progesterone are the hormonal prerequisites for menstruation (2). The characteristics of the endometrial changes (including the extensive changes observed in the endometrial vasculature) associated with the withdrawal of progesterone and menstrual bleeding suggest an involvement of vasoactive local mediators. The evidence that prostaglandin (PG) activity in the endometrium is modulated by progesterone, and the widely recognized vasoactive properties of PGs, make them prime candidates for mediators of progesterone action on the endometrium (3).

PGs are synthesized from arachidonic acid (AA); thus, the liberation of AA from precursors (present as membrane-bound phospholipids) by the action of phospholipase A_2 (PLA_2) is one of the first steps (and a rate-limiting step) in PG synthesis. AA is then converted to prostanoides (including PGE_2, $PGF_{2\alpha}$, and PGI_2) by the actions of cyclooxygenase (COX). In the endometrium, PGs are not stored but are immediately synthesized and released and metabolized to inactive metabolites by PG 15 dehydrogenase (PGDH) enzyme.

The antiprogesterone compound mifepristone (RU 486; Roussel Uclaf, Paris, France) is a synthetic 19-norsteroid with a specific high affinity for binding to the progesterone receptor (PR). It blocks the biological effects of progesterone by binding with high affinity to the PR (4). In the midluteal phase, mifepristone, at a single dose of 50–800 mg, induces menstrual bleeding within 72 h (5, 6). Luteolysis was incomplete in two thirds of the subjects, and they experienced a further episode of vaginal bleeding at the expected time of menses. In the remainder, there was complete luteolysis, with only one episode of bleeding. Thus, the vaginal bleeding observed after mifepristone, without a decrease in the circulating progesterone values, seems to be attributable to a direct effect on the endometrium. It has been suggested that the menstrual bleeding induced by mifepristone in midluteal phase is attributable to a direct effect on endometrial vessels (7). After treatment with 50 mg mifepristone in the midluteal phase (d 20–23), there is a significant reduction in the capillary luminal area and diameter associated with degenerative changes in the endothelial cells, which preceded the menstrual shedding. These changes do not always accompany regressive changes in the adjacent stroma. This effect of mifepristone on the endometrium (at a time when the en-

Abbreviations: AA, Arachidonic acid; AR, androgen receptor; COX, cyclooxygenase; ER, estrogen receptor; LH+8, d 8 after the onset of the urinary LH surge; PG, prostaglandin; PGDH, prostaglandin 15 dehydrogenase; PLA_2, phospholipase A_2; PR, progesterone receptor; RT, room temperature.

dometrial PR level is relatively low) is poorly understood and has been hardly investigated.

The endometrial effects of antigestogens given in the early-luteal phase have been extensively investigated (8–11). In the early-luteal phase, mifepristone inhibits progesterone-induced down-regulation of PR and estrogen receptors (ERs), while antagonizing the progesterone action on endometrial markers such as PGDH, which are known to be progesterone-dependent (12, 13). Moreover, PGDH has been postulated as a useful marker of the closure of the implantation window, and the effect of midluteal administration on such markers might add to our current understanding of potential contraceptive actions of mifepristone.

Our study investigated the mechanism of mifepristone-induced vaginal bleeding in the endometrium from 16 healthy women with regular cycles. The endometrial biopsies were performed between 0 (control) and 6-48 h after midluteal phase administration of a single dose of 200 mg mifepristone. We examined the expression and the distribution of sex steroid receptors in the endometrium and the expression of PGDH, and inducible PG synthesizing enzyme COX-2. Alterations in the expression of such progesterone-dependent proteins may widen our understanding of the mechanism by which mifepristone induces endometrial bleeding in the midluteal phase.

Subjects and Methods

Subjects

Twenty healthy women with regular cycles (25- to 30-d duration; mean age, 34 yr; range, 26–45 yr) were recruited into a randomized, single center study with mifepristone. Women were either using a reliable nonhormonal method of contraception or were abstinent. All women underwent a comprehensive screening procedure before commencing the study. This consisted of a full medical history and routine physical and gynecological examination, together with measurement of blood pressure, pulse, height, and weight. In addition, a venous blood sample was taken for full blood count, serum biochemistry, and liver function. These blood tests were repeated at the end of the study. All women kept a menstrual diary card and recorded all vaginal bleeding experienced during the study period and in the following cycle, and the day in which they identified an LH surge using urinary dipsticks.

We also studied the endometrial samples from four women taking part in a separate study, which evaluated the secretory endometrium. Those women also used the same type of urinary dipsticks to identify the urinary LH surge, and the biopsies were collected 7 or 8 d after the first day of the urinary LH surge. Because these women did not receive any treatment, the four biopsies were included in our control group. Therefore, the total number of biopsies analyzed in the control group was seven.

Lothian Research Ethics Committee (Institutional Review Board) approved the study, and informed written consent was obtained from each woman.

Study design

The women were monitored over two consecutive cycles: a treatment cycle, and a follow-up cycle. The women were allocated, at random, to 1 of 5 groups, depending on the timing of the biopsy, *i.e.* 6, 24, 36, or 48 h after a 200-mg mifepristone treatment on d 8 after onset of urinary LH peak (LH + 8). The control group had a biopsy but no treatment. Each of the 20 women in the study were allocated to the next consecutive study number in the randomization list before commencing the study. The randomization list was produced using the Statistical Package for Social Scientists (SPSS, Inc., Chicago, IL), such that each study number was randomly assigned to 1 of 5 groups in the study. The list was balanced after each block of 5.

Women used detection kits to detect the LH surge in a first sample of urine. On the occasion when the endometrial biopsy was taken, a blood sample was also collected for serum progesterone measurement by RIA.

Four women were subsequently withdrawn from the study: 1 because of previously undiagnosed cervical stenosis, which made endometrial biopsy difficult; in 1 woman, RIA could not confirm the self-detected LH peak; and the endometrial samples were inadequate for analysis in 2 other women. Therefore, we analyzed the endometrial samples in 13 women after taking mifepristone in the midluteal phase (n = 3, at 6 h; n = 4, at 24 h; n = 3, at 36 h; n = 3, at 48 h after mifepristone). Three samples from our original control group plus the above mentioned 4 additional samples from a separate study made a total number of 7 in the control group.

Detection of the urinary LH peak

The timing of the urinary LH surge were detected by the subjects themselves, using a commercially available LH detection kit (Oviquick; Unipath, Bedford, UK), which they used according to the manufacturer's instructions. The self-detected urinary LH peak was subsequently confirmed by RIA (MAI Aclone Kit; Biostat-Diagnostics, Stockport, Cheshire, UK).

Serum progesterone

A blood sample was collected immediately before the endometrial biopsy in all women, stored, and later assayed for progesterone. Serum progesterone measurements were done by using the Coat-A-Count progesterone procedure [solid-phase RIA; Diagnostic Products (UK) Ltd., Glyn Rhomwy, Llanbersi, Caernarfon, Gwwyedd, North Wales, UK.

Endometrial biopsies

Endometrial biopsies were obtained using a Pipelle endometrial sampling device (Prodimed, Neuilly-en-Thelle, France) and were fixed immediately in 4% paraformaldehyde for 24 h, routinely processed, and embedded in paraffin, and sections were cut to 5-μm thickness. All tissue samples were labeled with a code number for anonymity; and, except for this number, the mounted sections did not contain any other information.

Immunohistochemistry

Immunohistochemical staining was performed for immunolocalization of: 1) PR, with a 1:40 dilution of mouse monoclonal antihuman PR antibody (0.88 μg/ml protein; Novocastra Laboratories, Newcastle upon Tyne, UK); 2) ER, with a 1:400 dilution of mouse monoclonal antihuman ER antibody ER1D5 (0.58 μg/ml protein; DAKO Corp. Laboratories, High Wycombe, UK); 3) androgen receptor (AR), with a 1:480 dilution of monoclonal mouse antihuman AR antibody (F-39; BioGenex Laboratories, Inc. antibody, A Merarini Diagnostics, Berkshire, UK); 4) PGDH, with a 1:3000 dilution of rabbit polyclonal antibody (Dr. H. H. Tai, University of Kentucky, Lexington, KY); and 5) COX-2, with a 1:600 dilution of goat polyclonal antihuman COX-2 antibody (0.3 μg/ml protein; Santa Cruz Biotechnology Inc., Santa Cruz, CA).

Immunohistochemistry procedures

All protocols were optimized to determine the correct conditions for maximum specific staining, and all sections used as negative controls (where the primary antibody was replaced with nonimmune IgG of the same species and concentration) did not show immunostaining. Each immunostaining procedure was performed in a single run. The immunohistochemical technique used was as follows:

Five-micrometer paraffin sections were dewaxed in Histoclear (National Diagnostics, Yorkshire, UK) and rehydrated in descending concentrations of ethanol to dH20. After a 10-min wash in 0.01 M PBS (pH 7.4–7.6; PBS tablets, Sigma, Dorset, UK), antigen retrieval was carried out as follows: Sections to be stained for PgR and ER were microwaved for 10 min in 0.01 M sodium citrate buffer (pH 6) while those for AR and COX-2 were pressure-cooked in 0.01 M sodium citrate (pH 6) for 5 min and 2 min, respectively (Tefal, Nottingham, UK). An antigen retrieval

step was not required to expose the PGDH epitope. Sections were washed in PBS, and endogenous peroxide activity was blocked by incubation in 3% hydrogen peroxide in distilled water for 10 min (PR, ER, AR, PGDH) or 3% hydrogen peroxide in methanol for 30 min (COX-2). After a 10-min wash in PBS, an endogenous biotin blocking step was carried out for AR and COX-2, where sections were incubated sequentially in avidin then biotin for 15 min each at room temperature (RT) (Vector Laboratories, Inc., Peterborough, UK). After a further 10-min wash, sections were incubated in normal horse serum (Vector Laboratories, Inc.) for PR, ER, and AR or in 20% normal goat serum for PGDH, and in 20% normal rabbit serum for COX-2 (Diagnostics Scotland, Edinburgh, UK), all for 20 min at RT. The primary antibody was then added at the dilutions stated above. Sections were incubated for 60 min at 37 C for PR, ER, and COX-2 and overnight at 4 C for AR and PGDH. After washing in PBS with Tween 20, biotinylated horse antimouse antibody, followed by an avidin biotin horseradish peroxidase complex (ABC, Vectastain Elite; Vector Laboratories, Inc.) was added for 60 min for ER and AR, and 30 min for PR at RT. Biotinylated goat antirabbit and horse antigoat (Vector Laboratories, Inc.) were added for PGDH and COX-2, respectively, for 30 min, followed by the ABC complex for 60 min (PGDH) and 20 min (COX-2) at RT. Staining was visualized by incubation in 3,3′ Diaminobenzidine (DAB; DAKO Corp. Laboratories). Sections were then counterstained using Harris' Hematoxylin (Pioneer Research Chemicals Ltd., Essex, UK), dehydrated, and mounted in Pertex (Cellpath, Hemel Hempstead, UK).

Scoring and immunohistochemistry analysis

We employed a semiquantitative subjective scoring system to evaluate the intensity and the localization of immunoreactivity in entire tissue sections. Previously, we have reported that the immunostaining patterns in endometrial sections measured by the subjective semiquantitative scoring showed an almost perfect correlation with that measured objectively by computerized image analysis (14). Therefore, the less-time-consuming, semiquantitative scoring system provides a valid score suitable for graphical presentation.

Two independent observers, using light microscopy, visually assessed all coded sections. The two separate scores were then compared to obtain a more objective final score for each section. Once the final score had been agreed for all sections in the five-immunostaining runs, the code was broken. Afterwards, the final immunostaining scores were analyzed by the respective groups.

The immunostaining intensity of the steroid receptors (PR, ER, and AR) were scored using a four-point scoring scale, where the intensity of staining was assigned as 0 = none, 1 = weak, 2 = distinct, and 3 = strong. However, the staining intensity of PGDH and COX-2 showed a narrow range; and therefore, we adapted a three-point scoring scale, where the score of zero = an absence of immunoreactivity, 1 = faint immunoreactivity; and 2 = strong immunoreactivity.

Statistical analysis

Originally, the sample size was determined to include 5 women in each of 4 groups at 6, 24, 36, and 48 h after mifepristone. However, because of a number of reasons listed above, only 13 biopsies after treatment were available. Because mifepristone induced bleeding by 36–48 h in all women, a preliminary analysis was performed to determine the appropriate statistical test for analysis of the data. The mean staining intensity scores between 6-h and 24-h groups and between 36-h and 48-h groups showed no significant difference; hence, the 13 samples after treatment with mifepristone were analyzed in 2 groups: group 1 (6–24 h after mifepristone, n = 7), and group 2 (36–48 h after mifepristone, n = 6). Comparisons between these 2 groups were tested by nonparametric Kruskal-Wallis ANOVA test and the Dunn's multiple-comparisons test because they were discontinuous data sets.

Results

All women reported vaginal bleeding commencing 36–48 h after taking mifepristone. Four women in group 2 (one woman after 36 h, and three women after 48 h) had already started to bleed at the time the endometrial biopsy was taken; the others started bleeding after the biopsy. The bleeding lasted for 12–72 h, and all but three women reported a second bleed at the time of the expected menses. In these three women, a second episode of bleeding occurred approximately 4 wk later.

The concentration of progesterone was significantly lower at the time of biopsy in the women treated with mifepristone than in the control women (13.2 nM *vs.* 34.8 nM, $P = 0.001$). However, the levels were still significantly higher than those found during the follicular phase.

Intense PGDH immunoreactivity was observed in the cytoplasm of predominantly glandular epithelium (with a lesser degree of staining in the stromal cells) in all midluteal-phase control sections (Fig. 1A). The abundance of PGDH-positive immunostaining clearly declined, to be virtually absent by 36–48 h in both glands and in stroma (Fig. 1B). The difference in PGDH-staining scores between the control group and 36- to 48-h group were statistically significant ($P < 0.05$) (Fig. 2A).

In all samples (treated and untreated), COX-2 staining was localized predominantly in the endometrial glands, with no (or barely detectable) staining in the stroma (Figs. 1C and 2B). Untreated endometria in the midluteal phase showed minimal staining for COX-2 in the glandular cellular compartments. After mifepristone, a significant increase in immunoreactivity was apparent at 36–48 h (Figs. 1D and 2B) ($P = 0.0018$).

Steroid receptor immunostaining

As expected, there was weak staining for PR in the nuclei of stromal cells in the control samples, with minimal ER staining. AR staining was confined to stroma. There was no change in intensity or distribution of staining for steroid receptors after mifepristone (data not shown).

Discussion

Endometrial shedding and vaginal bleeding are observed after withdrawal of progesterone (for example at luteal regression) from an estrogen-primed endometrium that is subsequently exposed to progesterone (2). Similar bleeding is also seen after the pharmacological withdrawal of progesterone after administering the antiprogesterone, mifepristone, in the luteal phase of the cycle. While the endometrial morphology exhibits a marked sensitivity to mifepristone, with a 0.5-mg daily dose being the threshold dose for delay in endometrial maturation (15–17), in general, higher doses (in excess of 10 mg) are required to produce endometrial shedding and menstrual bleeding.

All women in our study reported vaginal bleeding commencing at 24–48 h after taking 200 mg mifepristone in the midluteal phase. In 13 out of the 16 subjects, this was followed by a second bleed of normal character at the expected time of the next menses. Although mifepristone significantly depressed the serum progesterone value in all women, the occurrence of a second bleed in the majority suggests only a partial luteolysis and a direct effect of mifepristone on the endometrium (18).

The current understanding advocates a central role for PGs as a trigger mechanism for menstruation (2, 3, 19, 20). The

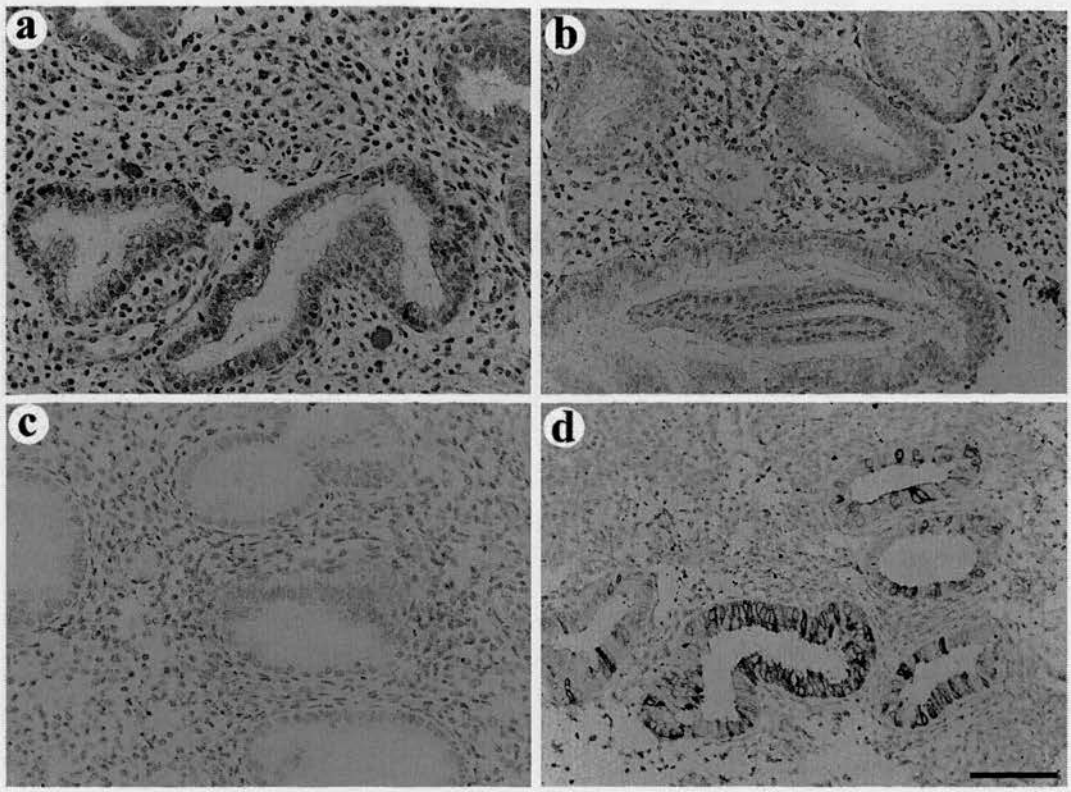

FIG. 1. A, PGDH immunostaining in midluteal-phase endometrium, demonstrating positive immunoreactivity in the glands and stroma. B, Endometrium, biopsied 36 h after administration of mifepristone, on d LH+8 of cycle. Note the decrease in immunoreactivity in the glandular and stromal compartments. C, COX-2 immunostaining in an endometrial biopsy collected in the midluteal phase. Negligible immunostaining in glands and stroma. D, COX-2 immunostaining in endometrium, collected 36 h after administration of mifepristone, on d LH+8. Note the increase in immunoreactivity in the glandular cytoplasm. *Scale bar*, 50 μm.

concentration of PG in any tissue is related to its rate of synthesis and metabolism, and the variation in the endometrial release of PGs at different stages of the menstrual cycle suggests an ovarian hormonal influence. The two key enzymes that control the endometrial PG synthesis (PLA_2 and COX) seem to be under the influence of progesterone. COX enzyme exists as two isoforms produced by two different genes (COX-1 and COX-2). COX-1 is constitutively expressed, whereas COX-2 expression is modulated by a variety of stimuli and may be inhibited by progesterone in the endometrium (21). PLA_2 also seems to be present in the endometrium in two different isoforms: the calcium-dependent and inducible $PLA_{2(i)}$, which is localized in the endometrial glands; and the calcium-independent $PLA_{2(ii)}$, which is predominantly confined to the stroma (22). Conversely, PGDH metabolizes PGs to inactive metabolites, and this enzyme is induced by progesterone (12, 13, 23). The increase in PGDH activity in a secretory endometrium that occurs in response to the rising levels of progesterone in the luteal phase (12) can be prevented by administration of antigestagens shortly after ovulation (11). Thus, progesterone is responsible for stimulating PGDH and suppressing COX-2.

In our study, treatment with mifepristone in the midluteal phase resulted in a decreased PGDH and an increased COX-2 expression in the endometrial glands, which was apparent at 36 h after mifepristone. This effect would be expected to be synergistic in increasing endometrial PGs (because of a synchronized suppression of metabolism, with an augmentation in the synthesis) and would lead to increased uterine activity and menstrual bleeding (2). In addition, progesterone withdrawal mediates the degradation of the endometrial extracellular matrix by inducing matrix metalloproteinases (24).

Attempts have been made to demonstrate the effects of progesterone on the endometrial PGs activity both *in vitro* and *in vivo* studies. Progesterone seems to enhance the PG biosynthetic capacity of the secretory endometrium. This is demonstrated by the *in vitro* studies employing cell culture techniques and by maintaining endometrial explants in culture (25, 26). Conversely, progesterone has shown to suppress the release of PGs from the endometrium (26, 27). Studies *in vitro* have reported a reduction of both the estradiol-stimulated and the basal PG production by progesterone (26, 28). Furthermore, during pregnancy, when progesterone levels are high, basal endometrial PG production is also reduced (29). It had been suggested that this effect might involve the inhibitory effect of progesterone on the PLA_2 activity (22).

The withdrawal of progesterone from an endometrium that has been primed with progesterone and estradiol results in an increased COX-2 expression, whereas continuing exposure to progesterone is associated with low levels of COX-2 expression (30). Evidence for increased PG activity by antagonizing progesterone also comes from *in vitro* data, which showed a dose-dependent induction of PGF2α release

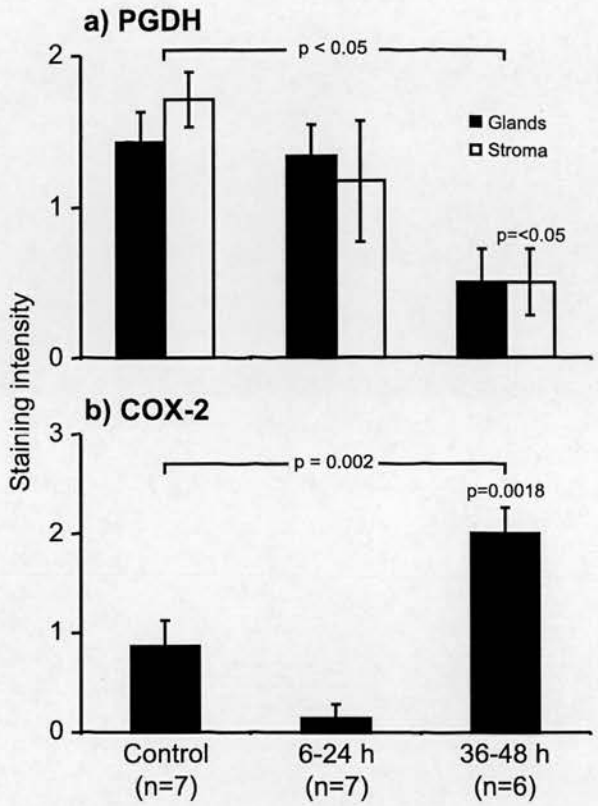

FIG. 2. Intensity of staining for PGDH (A) and COX-2 (B) in endometrial glands (■) and stroma (□) in the midluteal phase of the cycle before (control) and at times after administration of 200 mg mifepristone. *Bar*, Median and range.

from endometrial stromal cells (23) and also from the *in vivo* observation of increased uterine contractility after mifepristone, possibly attributable to increased PGs (31–33). This is further supported by the inhibition of glandular PGDH expression seen after administration of antiprogesterones in the early-luteal phase (10, 11) and during the early pregnancy (34).

A decrease in the uterine PGF2α release (33) and in the luminal expression of COX-2 had been reported after mifepristone in the early-luteal phase (35). However, in the early-luteal phase, progesterone values are relatively low; and, as a consequence, the PR and ER expression is maximal, whereas the converse is true for the midluteal phase of the cycle. Therefore, in the early-luteal phase, mifepristone may prevent the effects that are to be exerted by progesterone; whereas in the midluteal phase, it may antagonize the actions of progesterone, which, at that time, seems to be the suppression of PGs release.

Our results: are consistent with previous reports (10, 21) that demonstrated localization of PGDH and COX-2 in the glandular epithelium, and also support the *in vitro* evidence that glands are the main site for PG synthesis (36, 37).

There was no significant change in the level of immunoreactivity of AR, ER, or PR after mifepristone. When given immediately after ovulation, mifepristone and onapristone prevent the progesterone-induced down-regulation of PR and ER, which normally occurs during the luteal phase (10, 38, 39). In the midluteal phase, the levels of ER and PR are already low and were unchanged at 48 h after mifepristone administration. AR staining was mainly confined to the stromal compartment and remained unchanged after mifepristone (40). In contrast, when mifepristone was given in the early-luteal phase, there was strong immunostaining for AR in the glands (41). The factors regulating the spatial and temporal expression of AR in the endometrium and its physiological role are not fully understood.

The distinct endometrial effects seen after the midluteal administration of mifepristone add to our understanding of the mechanism of menstruation. There is overwhelming evidence that PGs are involved in the process of normal menstruation (Reviewed in Ref. 3). Our results show a down-regulation of PGDH expression and a simultaneous up-regulation of COX-2 expression after administering mifepristone in the midluteal phase. Therefore, we conclude that mifepristone induces endometrial bleeding, in the midluteal phase, by a mechanism involving both PGDH and COX-2 to increase local PG levels in the endometrium.

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References

1. **Corner GW, Allen WM** 1929 Physiology of the corpus luteum-II: production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. Am J Physiol 88:326–329
2. **Critchley HOD, Kelly RW, Brenner RM, Baird DT** 2001 The endocrinology of menstruation—a role for the immune system. Clin Endocrinol 55:701–710
3. **Baird DT, Cameron ST, Critchley HOD, Drudy TA, Howe A, Jones RL, Lea RG, Kelly RW** 1996 Prostaglandins and menstruation. Eur J Obstet Gynecol Reprod Biol 70:15–17
4. **Spitz IM, Bardin CW** 1993 Clinical pharmacology of RU 486—an antiprogestin and antiglucocorticoid. Contraception 48:403–44
5. **Schaison GM, Lestrat N, Reinberg A, Baulieu EE** 1985 Effects of the antiprogesterone steroid RU 486 during midluteal phase in normal women. J Clin Endocrinol Metab 61:484–489
6. **Shoupe D, Mishell Jr DR, Lahteenmaki P, Heikinheimo O, Birgerson L, Madkour H, Spitz IM** 1987 Effects of the antiprogesterone RU 486 in normal women. I single dose administration in the midluteal phase. Am J Obstet Gynecol 157:1415–1420
7. **Johannisson E, Oberholzer M, Swahn M-L, Bygdeman M** 1988 Vascular changes in the human endometrium following the administration of the progesterone antagonist RU 486. Contraception 39:103–117
8. **Swahn ML, Bygdeman M, Xing S, Cekan S, Masironi B, Johannisson E** 1990 The effects of RU 486 administered during the early-luteal phase on bleeding pattern hormonal parameters and endometrium. Hum Reprod 5:402–408
9. **Gemzell-Danielsson K, Svalander P, Swahn M-L, Johannisson E, Bygdeman M** 1994 Effects of a single post ovulatory dose of RU 486 on endometrial maturation in the implantation phase. Hum Reprod 9:2398–2404
10. **Cameron ST, Critchley HOD, Buckley CH, Chard T, Baird DT** 1996 The effects of post ovulatory administration of onapristone on the development of a secretory endometrium. Hum Reprod 11:40–49
11. **Cameron ST, Critchley HOD, Buckley CH, Kelly RW, Baird DT** 1997 Effect of two antiprogestins (mifepristone and onapristone) on endometrial factors of potential importance for implantation. Fertil Steril 67:1046–1053

12. **Casey ML, Hemsell DL, Johnston JM, MacDonald PC** 1980 NAD-dependent 15-hydroxyprostaglandin dehydrogenase activity in human endometrium. Prostaglandins 19:115–122
13. **Greenland KJ, Jantke I, Jennatschke S, Bracken KE, Vinson C, Gellersen B** 2000 The human NAD+ dependent 15-hydroxyprostaglandin dehydrogenase gene promoter is controlled by Ets and activating protein-1 transcription factors and progesterone. Endocrinology 141:581–597
14. **Wang H, Critchley HOD, Kelly RW, Shen D, Baird DT** 1998 Progesterone receptor subtype B is differentially regulated in human endometrial stroma. Mol Hum Reprod 4:407–412
15. **Croxatto HB, Salvatierra AM, Croxatto HD, Fuentealba B** 1993 Effects of continuous treatment with low-dose mifepristone throughout one menstrual cycle. Hum Reprod 8:201–207
16. **Spitz IM, Croxatto HB, Robbins A** 1996 Antiprogestins: mechanism of action and contraceptive potential. Annu Rev Pharmacol Toxicol 36:47–81
17. **Gemzell-Danielsson K, Swahn M-L, Westlund P, Bygdeman M** 1997 Effect of low daily doses of mifepristone on ovarian function and endometrial development. Hum Reprod 12:124–131
18. **Swahn M-L, Johannisson E, Daniore V, de la Torre B, Bygdeman M** 1988 The effect of RU 486 administration during the proliferative and secretory phase of the cycle on the bleeding pattern hormonal parameters and endometrium. Hum Reprod 3:915–921
19. **Baird DT** 2000 Mode of action of medical abortion. J Am Med Womens Assoc 55:121–125
20. **Milne SA, Perchick GB, Boddy SC, Jabbour HN** 2001 Expression, localisation, and signalling of PGE2 and EP2/EP4 receptors in human nonpregnant endometrium across the menstrual cycle. J Clin Endocrinol Metab 86:4453–4459
21. **Jones RL, Kelly RW, Critchley HOD** 1997 Chemokines and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation. Hum Reprod 12:1300–1306
22. **Bonney RC, Franks S** 1987 Modulation of phospholipase A2 activity in human endometrium and amniotic membranes by steroid hormones. J Steroid Biochem 26:467–472
23. **Kelly RW, Healy DL, Cameron MJ, Cameron IT, Baird DT** 1986 The stimulation of prostaglandin production by two antiprogesterone steroids in human endometrial cells. J Clin Endocrinol Metab 62:1116–1123
24. **Lockwood CJ, Krikun G, Hausknecht VA, Papp C, Schatz F** 1998 Matrix metalloproteinase inhibitor expression in endometrial stroma cells during progestin-initiated decidualisation and menstruation related progestin withdrawal. Endocrinology 139:4607–4613
25. **Smith SK, Abel MH, Baird DT** 1984 Effects of 17 beta-estradiol and progesterone on the levels of prostaglandins F2 alpha and E in human endometrium. Prostaglandins 27:591–597
26. **Abel M, Baird DT** 1980 The effect of 17β-estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. Endocrinology 106:1599–1606
27. **Smith SK, Kelly RW** 1987 The effect of the antiprogestin RU 486 and ZK 98734 on the synthesis and metabolism of prostaglandins F2 alpha and E2 in separated cells from early human deciduas. J Clin Endocrinol Metab 65:527–534
28. **Kelly RW, Smith SK** 1987 Progesterone and antiprogestins, a comparison of their effect on prostaglandin production by human secretory phase endometrium and decidua. Prostaglandins Leukot Med 29:181–186
29. **Maathuis JB, Kelly RW** 1978 Concentrations of prostaglandins F2 alpha and E2 in the endometrium throughout the human menstrual cycle, after the administration of clomiphene or an estrogen-progestin pill and in the early pregnancy. J Endocrinol 77:361–371
30. **Critchley HOD, Jones RL, Lea RG, Drudy TA, Kelly RW, Williams ARW, Baird DT** 1999 Role of inflammatory mediators in human endometrium during progesterone withdrawal and early pregnancy. J Clin Endocrinol Metab 84: 240–248
31. **Norman JE, Wu XW, Kelly RW, Glasier AF, McNeilly AS, Baird DT** 1991 Effects of mifepristone *in vivo* decidual prostaglandin synthesis and metabolism. Contraception 44:89–98
32. **Gemzell-Danielsson K, Swahn M-L, Bygdeman M** 1990 Regulation of nonpregnant human uterine contractility. Effect of antihormones. Contraception 42:323–335
33. **Gemzell-Danielsson K, Hamberg M** 1994 The effect of antiprogestin (RU 486) and prostaglandin biosynthesis inhibitor (Naproxen) on uterine fluid PGF2α concentrations. Hum Reprod. 9:1626–1630
34. **Cheng L, Kelly RW, Thong KJ, Hume R, Baird DT** 1993 The effects of mifepristone (RU 486) on prostaglandin dehydrogenase in decidual and chorionic tissue in early pregnancy. Hum Reprod 8:705–709
35. **Marions L, Danielsson KG** 1999 Expression of cyclo-oxygenase in human endometrium during the implantation period. Mol Hum Reprod 5:961–965
36. **Lumsden MA, Brown A, Baird DT** 1984 Prostaglandin production from homogenates of separated glandular epithelium and stroma from human endometrium. Prostaglandins 28:485–496
37. **Smith SK, Kelly RW** 1988 The release of PGF2α and PGE2 from separated cells of human endometrium and deciduas. Prostaglandins Leukot Essent Fatty Acids 77:361–371
38. **Berthois Y, Brux JD, Salat-Baroux J, Kopp F, Cornet D, Martin PM** 1991 A multiparametric analysis of endometrial estrogen and progesterone receptors after the postovulatory administration of mifepristone. Fertil Steril 55:547–554
39. **Maentausta O, Svalander P, Gemzell-Danielsson K, Bygdeman M, Vihko R** 1993 The effects of an antiprogestin, mifepristone, and an antiestrogen, tamoxifen, on endometrial 17β-hydroxysteroid dehydrogenase and progestin and estrogen receptor during the luteal phase of the menstrual cycle; an immunohistochemical study. J Clin Endocrinol Metab 77:913–918
40. **Burton KA, Hillier SG, Habib FK, Mason JI, Critchley HOD, Slayden OD, Nayak NR, Burton KA, Chwalisz K, Cameron ST, Critchley HO, Baird DT, Brenner RM** 2001 Progesterone antagonists increase androgen receptor expression in the rhesus macaque and human endometrium. J Clin Endocrinol Metab 86:2668–2679
41. **Slayden OD, Nayak NR, Burton KA, Chwalisz K, Cameron ST, Critchley HOD, Baird DT, Brenner RM** 2001 Progesterone antagonists increase the androgen receptor expression in the rhesus macaque in human endometrium. J Clin Endocrinol Metab 86:2668–2679